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# Overwintering strategies of a guild of cereal aphid parasitoids along a climatic gradient

POR

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# Overwintering strategies of a guild of cereal aphid parasitoids along a climatic gradient

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## Título: Estrategias de hibernación de un gremio de parasitoides de áfidos de los cereales a lo largo de un gradiente latitudinal

**Palabras clave:** diapausa, plasticidad térmica, adaptación local, interacción hospedero-parasitoide efecto huésped

**Resumen:** Los insectos como pequeños ectotermos viven en una amplia gama de ambientes térmicos y tienen que adoptar diferentes estrategias para resistir condiciones desfavorables tales como: migración, entrar en diapausa o permanecer activos. A lo largo de esta Tesis, se han explorado diferentes impulsores ecológicos en parasitoides de áfidos a lo largo de un gradiente latitudinal en el valle centro-sur de Chile. Se evaluó si las condiciones ambientales a lo largo de este gradiente llevaron a una mayor incidencia de diapausa en latitudes altas (más frías) en comparación con latitudes bajas (más cálidas). Además, exploramos si la diapausa está regulada por estímulos bióticos (es decir, efecto del hospedero, disponibilidad del hospedero y la competencia materna). Se observó una ausencia de un claro gradiente de temperatura latitudinal norte-sur. Según la intensidad del invierno, las localidades muestreadas se clasificaron como áreas de invierno frío, templado o cálido. Se observaron niveles bajos de incidencia de diapausa a lo largo de todo el gradiente latitudinal.

Sin embargo, observamos cambios en la composición de las especies de áfidos y parasitoides, ya que sus niveles de abundancia relativa variaron entre las áreas climáticas, mostrando que la mayoría de los individuos adultos dentro del gremio de parasitoides están activos durante el invierno. Además, la diapausa en los parasitoides de áfidos estuvo influenciada por la especie hospedera atacada y por la percepción de escasez de hospederos en la diapausa de invierno. Asimismo, la competencia intraespecífica directa entre hembras de parasitoides en condiciones de verano se tradujo en un aumento de la descendencia en diapausa, sin embargo, este efecto no se observó en condiciones de invierno. Por lo tanto, la plasticidad del desarrollo y las respuestas evolutivas observadas ofrecen una explicación mecánica para la variación adaptativa de la historia de vida en los parasitoides de áfidos, lo que sugiere que el éxito de los parasitoides como agentes naturales depende en gran medida de la densidad del hospedero y el momento de las actividades estacionales.

## Title: Overwintering strategies of a guild of cereal aphid parasitoids along a latitudinal gradient

**Keywords:** Diapause, thermal plasticity, local adaptation, host-parasitoid interaction, host effect

**Abstract:** Insects as small ectotherms are known to live in a wide range of thermal climates and have to adopt different strategies to resist unfavorable conditions such as: migration, enter in diapause or remain active. Throughout this Thesis, different ecological drivers have been explored in aphid parasitoids along a latitudinal gradient in the central–south valley of Chile. We tested whether environmental conditions along this gradient led to a higher diapause incidence on high latitudes (colder) compared to low latitudes (warmer). In addition, we explored whether diapause is regulated by biotic stimulus (i.e., host effect, host availability, and maternal competition). We found an absence of a clear north-south latitudinal temperature gradient. Based on the intensity of winter, the sampled localities were categorized as either cold, mild, or warm winter areas. Low levels of diapause incidence were observed along the whole latitudinal gradient.

Nevertheless, we observed changes in the compositions of aphid and parasitoid species, as their levels of relative abundance varied among the climatic areas, showing that most adult individuals within the parasitoid guild are active during winter. In addition, diapause in aphid parasitoids was influenced by the attacked host species and from the perception of host scarcity in winter diapause. Likewise, direct intraspecific competition among female parasitoids in summer conditions has been translated into an increase of the diapause offspring, however, this effect was not observed in winter conditions. Thus, developmental plasticity and the observed evolutionary responses offer a mechanistic explanation for adaptive life-history variation in aphid parasitoids suggesting the success of parasitoids as natural agents depends strongly on host density and timing of seasonal activities.

# Part 1
# Introduction

## Chapter I: General introduction

## General background

The effects of the abiotic and abiotic aspects of the environment on biological parameters of organisms such as survival, growth, and reproduction, and the ways in which these effects are modified through seasonality, are receiving considerable attention by ecologists. Organisms, including insects encounter and respond to numerous abiotic and biotic stressors in the environment (Bijlsma and Loeschcke 2005; Freedman 2015). An environmental stressor is one in which the organism is not able to entirely compensate for the selective conditions that injure its performance or fitness (Angilletta et al. 2002). As ectotherms, insects have ecological requirements encompassing several specific conditions and resources such as climatic conditions and food (Chown et al. 2004). Hence, they respond in relative short time and low thresholds to abiotic factors and can be mediated through biotic interactions (Gabrys et al. 2008; Colinet et al. 2015).

Insects often must be able to survive predictable and recurring periods of environmental stress, such as cold and heat events. (Freedman 2015) It is widely known that environmental variability and seasonality have profound effects on the abundances, distributions, and life histories and that they are capable to change their activity depending on the conditions of the surrounding environment (Denlinger and Lee 2010; Abram et al. 2017) and temperature is the main determinant of survival in changing environments (Chapman et al. 2015). For an insect species to survive in any given geographic location, its life cycle must be tightly synchronized to all biotic and abiotic factors required for its development and reproduction. For instance, temperate insects face seasonally cyclical environmental stress (Sinclair et al. 2013). To cope with the different environmental conditions, insects use a wide variety of behavioral and physiological strategies that allow them to sense environmental changes and respond appropriately to these environmental variations such as migration, remaining active and reproduce or entering diapause (Masaki 1980; Tauber et al. 1986; Tougeron 2019; Tougeron et al. 2020). For some insects, migration is one strategy for escaping extreme environmental conditions (e.g., extreme temperatures) and has been shown to occur in a great variety of taxa (Wolda 1988; Saunders 2010). On the other hand, remaining active during unfavorable conditions (e.g., winter conditions) allows insects to complete their development and sometimes reproduce, but with the risk of reaching their minimal temperature survival threshold (Danks 1991). This strategy involves essential behavioral and physiological traits (e.g., chill coma, heat/cold stupor), where thermal tolerance plays an important role (Langer and Hance 2000; Sorensen et al. 2001; Denlinger and Lee 2010; Le Lann et al. 2011; Gibert et al. 2019). Another strategy employed by insects facing extreme environmental conditions is entering in diapause. Diapause is an adaptive strategy to overcome adverse environmental seasons such as frost events during winter (called winter diapause or hibernation) and when high temperatures and dry conditions are experienced during summer (summer diapause) (Masaki 1980; Denlinger et al. 2012). Diapause is an alternative life-history strategy defined as a state of suspended development or with low metabolic activity (Koštál et al. 2017). This strategy can be triggered by abiotic signals, especially changes in the photoperiod and temperature, that are detected before unfavorable conditions occur (Masaki 1980; Tauber et al. 1986; Denlinger and Lee 2010; Denlinger et al. 2012).

Adaptation to seasonal changes includes the ability to predict the upcoming gradual changes in environmental signals early enough to prepare for the harsh conditions (Saunders 2020). In temperate regions, cold seasons alternate with warmer seasons, also switching between rainy and dryer times (Wolda 1988; Johansson et al. 2020). Such climatic seasons tend to translate into seasonal activity patterns in many insects. Therefore, studying response across environmental gradients provides an expansive view of how insects adapt to spatial environmental heterogeneity (Danks 1991; Hoffmann and Hercus 2000; Gotthard 2001; Danks 2006). To evaluate environmental variability, there are two general assumptions. First, the latitudinal or/and altitudinal gradient in geographic range size is a consequence of climatic variability and, second, that there is a matching cline with the life-history and physiological traits of the organisms/individuals present along this gradient (Gaston et al. 1998). The responses of insects also need to be addressed in response to their biotic environmental stimuli that act at a certain time (Langer and Hance 2000; Tougeron et al. 2020). In addition, the diverse and often indirect effects of climate change on insects will be difficult to predict unless there is a good understanding of the way environmental effects impact trophic interactions (Tougeron et al. 2020).

In this thesis, the winter parasitoid-aphid composition and overwintering strategies of parasitoids in a latitudinal gradient in Chile, Parasitoids have evolved several adaptations to synchronize their life cycle with their host, where diapause expression is subject to environmental conditions (photoperiod and temperature) and other cues such as host scarcity, maternal effect may play a marginal role (Brodeur and McNeil 1989; Polgár and Hardie 2000; Tougeron et al. 2017). This strategy may lead to a mismatch between pests and natural enemies in space and time, with potential positive or negative impact on biological control (Gariepy et al. 2015; Tougeron et al. 2017). Therefore, improving the knowledge of factors inducing diapause in parasitoids is important if we aim at improving biological control effectiveness.

## Environmental stress

Understanding and explaining the pressures underlying differences in performance within and between species has been an important topic in Ecology and Evolution. Organisms must deal simultaneously and constantly with anthropogenic and natural environmental variations to effectively achieve their optimal fitness for a given context. It has been well documented that local environmental conditions affect organisms in different ways either by acting as constraints or by causing detrimental effects on their associated functions and processes (Enders and Miller 2016). Environmental stress may be described as a force shaping adaptation and evolution in changing environments and may lead to a sharp reduction in survival and reproduction of affected populations (Schulte et al. 2014). A variety of biotic and abiotic factors are included in this definition, ranging from physical stressors (e.g., climate) (Mugabo et al. 2019), chemical stressors (e.g., insecticides) (Liess et al. 2021) and biological stressors (e.g., trophic interactions) (Chattopadhyay et al. 2019). In natura, all these factors fluctuate and interact, and their effects have been recognized in diverse areas of research as physiology, behavior, biological control, with an increasing number of studies because of climate change (van Baaren et al. 2010; Williams et al. 2015).

Ectotherms during most of their life cycle, encounter simultaneously a considerable range of stressful stimuli that may threaten their fitness (Williams et al. 2015). Especially, temperatures have extensive biological implications for all organisms, since their biology is closely linked to the environmental temperature (Bijlsma and Loeschcke 2005; Huey 2010; Colinet et al. 2015). In addition, altered temperature regimes can also affect ectotherms indirectly by altering the quality of the biotic resources they utilize and/or their interactions with others organism. (Ragland et al. 2008; Fusco and Minelli 2010; Crutsinger et al. 2013; Van Dyck et al. 2015; Koštál et al. 2017).

Insects are among the most resilient, abundant and biodiverse ectotherms. They may be especially susceptible to thermal stress given that their physiology is limited by environmental temperatures (Porcelli et al. 2017). They have evolved varied traits and strategies allowing them to detect and survive in thermal stressing environments. Under some selective pressures, individuals with particular sets of traits may be favored which may lead to optimal survival and reproduction (Harrison et al. 2012; Colinet et al. 2015). Therefore, the understanding of insect response to thermal stress, provides useful lens through which physiology and ecology can be linked with the implications to predicting responses to climate change.

## Thermal stress: ecology of thermal adaptation

Temperature has been attributed a main role in the ecology and evolution of ectotherms and its effects extend from the molecular level to the whole organism (Angilletta Jr 2009). It is a key driver of organism distributions, meaning that temperature will have a vast impact on fitness and current species' ranges (Addo-Bediako et al. 2000; Vázquez and Stevens 2004; Vázquez et al. 2017). The importance of temperature in ectotherm performance (fitness) is well known (Angilletta et al. 2002; Angilletta and Dunham 2003; Terblanche et al. 2007; Angilletta Jr 2009).

In insects, the impact of temperature on the thermal performance is an important and direct biological consequence of climate variation and can be evaluated at different scales, since their basic physiological functions (i.e., growth, reproduction) are strongly influenced by environmental temperature (Sinclair et al. 2003; Turnock and Fields 2005). They respond to the whole thermal regime, which is characterized by patterns of absolute temperatures, seasonal variability and rates of change, together with other environmental components such as photoperiod (Atkinson 1994; Sinclair et al. 2003; Danks 2007). In temperate regions, thermal ecology is a good proxy of fitness of an organism because environmental temperature has a direct impact on all aspects of insect performance. This can be described by thermal performance curves (TPCs) that show a gradual increase in performance from a critical thermal minimum ($T_{min}$) where fitness is zero, until reaching an optimal temperature ($T_{opt}$) where performance is the highest, before decreasing towards a critical thermal maximum ($T_{max}$) (Johansson et al. 2020) (Figure 1.1).

Thermal stress can have rapid lethal or sub-lethal effect in insects, which has been widely reported (Chown et al. 2004; Danks 2007; Kellermann and van Heerwaarden 2019; Johansson et al. 2020). However, understanding the impact of thermal stress on the development and reproduction of surviving insects could provide important basic information for insect ecology (Perez and Aron 2020). Some studies of insect cold tolerance have addressed sublethal effects of cold injury, including those that may reduce fitness (Atkinson 1996; Bale 2002; Angilletta et al. 2002). Species inhabiting low temperature environments must either be able to tolerate or avoid these conditions to survive (Bale 2002). For instance, it has been assumed that the close relationship between temperature and development, is vital for understanding a diversity of biological processes (Honěk and Kocourek 1990).

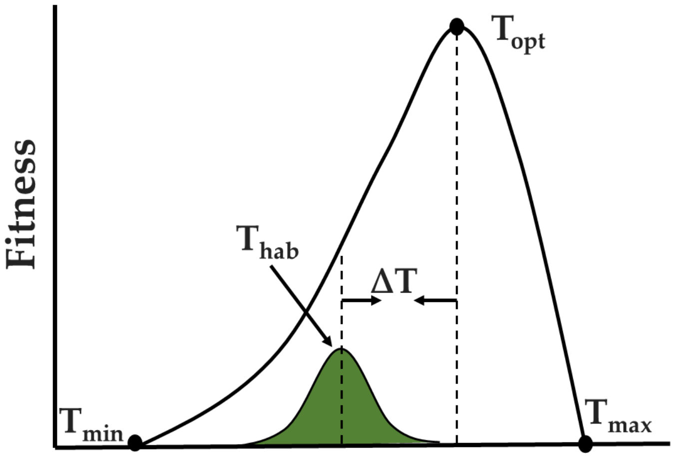

**Figure 1.1.** Thermal performance curves of ectotherms in temperate environments. $T_{min}$ and $T_{max}$ represent the minimum and maximum temperature at which organisms can perform, and $T_{opt}$ is the optimal temperature for performance. The green curve under the thermal performance curve is the temperature that the organism is exposed to during an average time and the average is represented as $T_{hab}$. $\Delta T$ is the distance between $T_{hab}$ and $T_{opt}$ (Johansson et al. 2020).

Tolerance to extreme temperatures is possibly among the most important trait shaping the fundamental niche of insects (Addo-Bediako et al. 2000; Angilletta Jr 2009). Insect also have different levels of physiological sensitivity and may respond differently to cold stress. The effects of temperature on physiological processes strongly influence the ability of insects to carry out activities for their survival (MacMillan and Sinclair 2011; Colinet et al. 2015). In the last years, critical thermal limits (CTL) (Box 1) have received much attention since they provide insights into the way in which climate shapes variation in the ecology, distribution, and evolution of species (Terblanche et al. 2007; Mutamiswa et al. 2019). CTLs are considered as ecologically relevant because they provide an indication of the activity range for a population under acute exposure conditions (Chown 2001; Chown et al. 2004; Terblanche et al. 2007).

## BOX 1. Critical thermal limits (CTLs)

The Critical thermal limits are described by the $CT_{Min}$ and $CT_{Max}$ and include also the preferred temperature (Le Lann et al. 2011). For testing theory about environmental limitations at low temperatures, critical thermal minima ($CT_{Min}$) have been used as measures of fitness and performance in a wide variety of species. $CT_{Min}$ determination involve cooling an organism from a starting temperature until physiological failure (e.g., loss of muscle response) (Terblanche et al. 2007; MacMillan and Sinclair 2011; Le Lann et al. 2011). When insects are cooled, two main kinds of response were identified corresponding to two different definitions: Locomotion trouble is used because it is an ecologically relevant parameter. The animal is still able to walk but without coordination, which corresponds to the loss of coordinated muscle functions ($CT_{Min1}$), whereas Chill coma ($CT_{Min2}$) is a reversible state where neuromuscular transmission and movement cease that include a series of behavioral and physiological events (including $CT_{Min1}$). In many research with insects, it has been observed that $CT_{Min1}$ and chill coma have been used as synonymous, however this is incorrect. (Figure 1.2) (Terblanche et al. 2007; Hazell and Bale 2011).

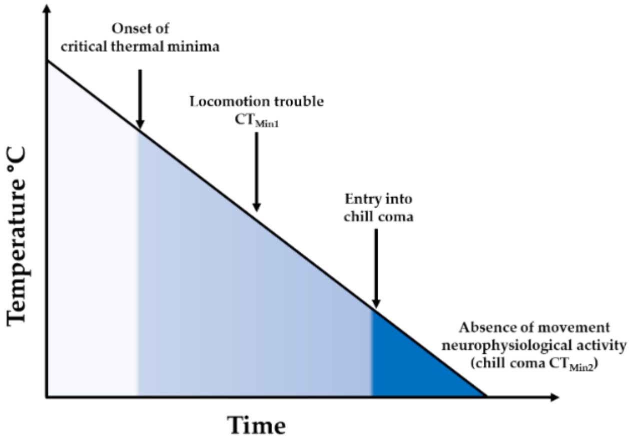

**Figure 1.2.** Diagram of behavioral and physiological thresholds measured. Onset of critical thermal minima is highlighted in light blue, beginning at the temperature at which locomotor efficiency is first compromised (locomotor trouble $CT_{Min1}$). The threshold temperatures at which spontaneous movements and coordination are lost (entry to chill coma. Chill coma ($CT_{Min2}$) the physiological state is highlighted in dark blue (Modified from Hazell and Bale, 2011).

## Thermal stress effects on life-history traits and fitness

Ectotherms experience repeated environmental thermal stress exposure on multiple time scales in natural environments (Marshall and Sinclair 2018). Selection acts on the ability of ectotherms to function optimally within the range of temperatures that they regularly face to minimize fitness costs during exposure to suboptimal temperatures (Bale 1987; Angilletta Jr 2009). The effects of thermal stress can carry over different life stages and can even have a transgenerational effect (Tougeron et al. 2020).

Fluctuating temperatures (high or low ones) can allow development outside the temperatures where it would normally occur with consequences on performance (fitness) (Colinet et al. 2015) (Colinet et al. 2015). As measuring lifetime reproductive success is often difficult, traits positively correlated with reproductive success are often measured (e.g., morphology, development duration, longevity, egg load, or mating success) (Roitberg et al. 2001). For instance, it has been known that morphology (i.e., body size, shape), integrate the stress experienced during development and can thus provide a measure of developmental stability in changing environments (Colinet et al. 2015). On the other hand, it has been assumed the close relationship between temperature and development, vital for understanding a diversity of biological processes (Honěk and Kocourek 1990). At low temperatures, slower development appears to be the norm when the minimum temperature is not detrimental (Ratte 1984). The temperature-size rule predicts that development at low temperatures should result in larger insects (Atkinson 1994). These responses depend on the thermal mean that is used and its proximity to developmental thresholds (Colinet et al. 2015). Longevity is another trait affected by thermal stress in many species. For instance, it was observed in *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) where its life span declined significantly due to increasing temperature (Butler and Trumble 2010). Similar response was recorded in *Drosophila melanogaster* Meigen (Diptera, Drosophilidae) after cold stress exposition (Koštál et al. 2019). When the temperature is close to the lethal thermal limits, insects can harbor thermal injuries, which result in a decreased fecundity (Melicher et al. 2021). Fecundity is a central component of fitness and can thus be used as a proxy to assess fitness consequences at low temperatures (Le Ralec et al. 2010; Hahn and Denlinger 2010). For instance, cold exposures (below 0◦C) decrease reproductive output in *Drosophila melanogaster* (Marshall and Sinclair 2010). Also, it has been reported that heat stress has detrimental effects on oocytes and ovarian development in females which could lead to a decrease in egg production in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Noor-Ul-Ane et al. 2018).

In addition, exposure to low temperatures may cause cellular damage, metabolism, and physiological perturbations (Zamorano et al. 2017). Cold exposure can induce chilling injuries that lead to a complex physiological disruption such as loss of ions and water homeostasis, which result in the disorder of neuromuscular functions, that can ultimately cause death (Overgaard and MacMillan 2017). Recent studies have also shown substantial levels of DNA damage in hemocytes when insects were heat and cold stressed, suggesting temperature stress can affect DNA integrity (Lubawy et al. 2019).

## Different types of responses to thermal stress

In insects, in addition to variability in environmental temperature and duration of the growing season, their responses to environmental stress are an important key to cope with different environments (Addo-Bediako et al. 2000; Hoffmann et al. 2003; Kellermann and van Heerwaarden 2019). In general, insects' response to environmental stressors depends on their intensity and duration. According to duration, stress is classified as acute (i.e., short-duration), or chronic (i.e., long-duration), with acute stressors being more frequent than chronic stressors (Schulte et al. 2014). While some species expand rapidly and respond successfully to stressful environments, others tend to fail and consequently suffer population decline in response to stressful environments (Angilletta Jr 2009; Denlinger and Lee 2010; Colinet et al. 2015). To live successfully at low temperatures there is an integration of behavioral, developmental, physiological and biochemical syndrome of traits (Tauber et al. 1986; Danks 1991; Sinclair et al. 2003), which are shaped by genetic adaptations and/or plastic responses (Sandoval-Castillo et al. 2020). These traits allow organisms to maintain steady internal conditions required for physiological functioning (Figure. 1.3) through different mechanisms such as plasticity or adaptation.

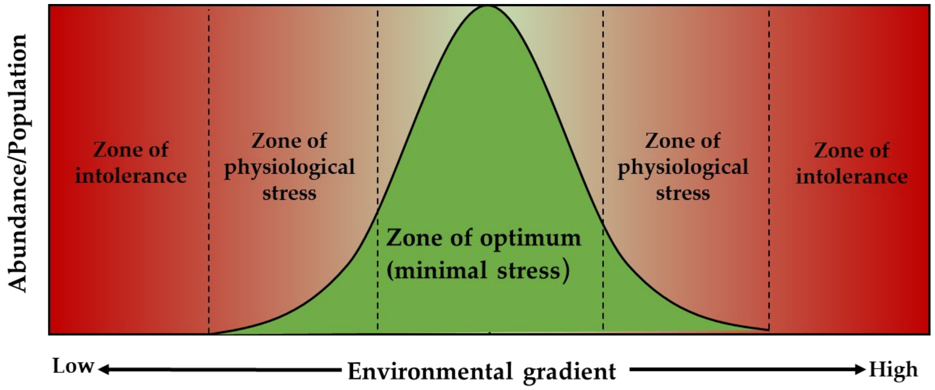

**Figure 1.3.** Ecological tolerance curve. The green shaded area represents the population frequency distribution of one species along the environmental gradient (e.g., cool to warm temperature) (Faith and Lyman 2019).

### *Local adaptation to temperatures*

The ability of natural populations to respond to environmental changes will depend on the level and type of perturbation the organisms experience, and on their capability to respond to them. Temperature effects extend from the molecular level to the whole organism (Angilletta Jr 2009). Genetic variation for physiological and life-history traits inside natural populations is shaped by a random genetic drift, flow genes and natural selection or a combination of these and maintained by different process such as mutation and recombination (Logan et al. 2014; Lancaster et al. 2016). Understanding the extent to which exposure to extreme conditions impacts fitness traits is critical to recognize the evolutionary potential and constraints on thermal adaptation (Porcelli et al. 2017). Insects that experience different thermal gradients may exhibit variation in adaptive responses to fluctuating temperatures (Colinet et al. 2015). Insects are able to adjust their physiology and behavior to maximize their fitness in extreme conditions (Chown 2001) through different mechanism such as plasticity (see below). Nevertheless, in the absence of broad gene flow, widely distributed species may be genetically structured into isolated populations. Divergent selective pressures in local environments can result in population differentiation according to their thermal biology, and their patterns of critical thermal limits (Overgaard and Sørensen 2008; Jackson et al. 2020).

Insects with broad and/or restricted distributions experience some degree of spatial and temporal environmental variation. This variation includes large-scale climatic gradients where temperature changes with latitude or elevation or a small-scale (microhabitats) (Tougeron et al. 2016; Kellermann and van Heerwaarden 2019). Populations are locally adapted when populations have the highest relative fitness at their local environment and lower fitness in other parts of the range (Heath et al. 1971; Porcelli et al. 2017; Marshall et al. 2020). Many studies have analyzed responses to thermal stress in insects and quantified local adaptation. In particular, critical thermal limits (CTLs) have received much interest to provide evidence of species distribution, ecology and evolution related to optimal thermal performance (García-Robledo et al. 2016), with many evidence from a variety of species demonstrating local adaptation to temperature ( see Sternberg and Thomas 2014 and references inside). For instance, adaptations have been observed on an evolutionary scale (multiple generations) in populations of drosophilid species (*Drosophila spp*) through differences both between and within species where the upper and lower thermal tolerance limits are correlated with the environmental gradient (Hoffmann et al. 2003). These results suggest that tolerance to extreme temperatures varies between species and populations of *Drosophila*, associated with the local temperature conditions experienced by the populations. Also, local adaptation was observed in winter diapause expression of aphid parasitoids where the incidence of diapause was higher in Canadian populations (harsh winter) than in French populations (mild winters) even when they experience French climate, suggesting a strong genetically determined local adaptation in harsh winter parasitoid populations (Tougeron et al. 2018d). Therefore, understanding the response of species to thermal challenges requires an understanding of their acute responses to environmental variability.

### *Plasticity as a response to environment variability*

In general, phenotypic plasticity can be defined as the capacity of a single genotype to exhibit variable phenotypes in different environments (Fusco and Minelli 2010; Gibert et al. 2019), it is common in insects and is often highly adaptive (Whitman and Agrawal 2009). Phenotypic plasticity can occur within a generation or over successive generations (transgenerational plasticity) (Ghalambor et al. 2015) and it is usually represented by the relationship between environment and phenotype for a given genotype called a *reaction norm* (Uller et al. 2020). A reaction norm is a function or series of functions defining the change in a genotype's phenotype across a changing environment (Figure 1.4), where genotypes inherit the genetic capability to produce each of the different phenotypes in the various environments (Nussey et al. 2007). Plasticity can affect the genotype of different ways. Phenotypic plasticity It could be passive, anticipatory, instantaneous, delayed, continuous, discrete, permanent, reversible, beneficial or detrimental (Whitman and Agrawal 2009).

Phenotypic plasticity could be crucial to enhance survival and explain variation in the success of species to resist a change in their environment (Sgrò et al. 2016; Abram et al. 2017; van Baaren and Candolin 2018). The role of behavioral and physiological plasticity in thermal adaptation has been widely recognized (Abram et al. 2017). This includes various developmental trajectories in response to specific environmental cues, and the ability of an individual to adjust its activity in response to environmental variations (Seebacher et al. 2015; Sgrò et al. 2016; Lafuente et al. 2018). Phenotypic plasticity may affect species distribution and vulnerability, it also may impact positively or negatively the ability to adapt to challenges arising in different environments with consequences on population persistence (Leonard and Lancaster 2020). For instance, it has been demonstrated that the physiological degree of cold hardening in *Drosophila suzukii* (Diptera, Drosophilidae) depend on the acclimation temperature to which the insects are exposed during its development (Stockton et al. 2018). In nature, predictable climatic variability favors the emergence of phenotypic plasticity, which plays an important role in the ecology and evolution of organisms (Fusco and Minelli 2010; Sgrò et al. 2016; Gibert et al. 2019). This suggests that organisms with more plasticity in how they respond to the environment may have better capability to survive in novel and/or changing environments (Gibert et al. 2019).

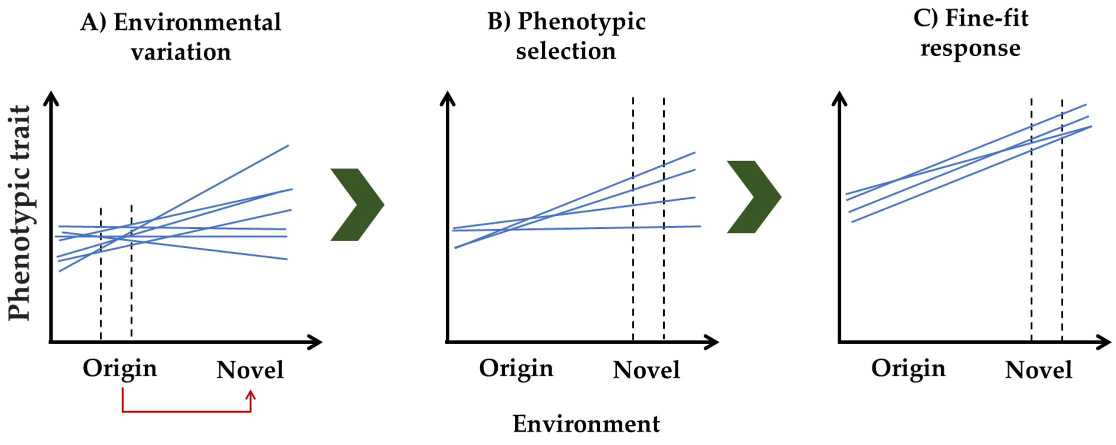

**Figure 1.4.** Phenotypic plasticity in terms of reaction norms: A) A group of genotypes adapted to their environment of origin is exposed to a novel environment, which results in the expression of phenotypes that were not observed in the original environment. B) Proxy of fitness differences between phenotypes results in the retention of a subset of the genotypes, which increases the mean fit between the phenotype and the new environment, and C) Fine-fit response of reaction norms of genotypes compared to the origin population. The blue lines represent the reaction norms of one genotype which show an increased in plasticity (Modified from Uller et al. 2020).

As environmental variability exerts selection pressure on fitness traits, phenotypic plasticity (adaptive and non-adaptive) and bet-hedging that is an evolutionary strategy that decreases the temporal variance in fitness at the expense of a lowered arithmetic mean fitness (Gianella et al. 2021) are expected (Joschinski and Bonte 2021) the latter more often in unpredictable environments. Adaptive plasticity evolves when the environmental cues are reliable and environments vary frequently (selection for plasticity is strong) (Acasuso-Rivero et al. 2019). Genetic and environmental correlations (genotype x environment interaction G x E) are themselves plastic and plastic responses can facilitate adaptation involving genetic change (Whitman and Agrawal 2009; Acasuso-Rivero et al. 2019), this is the first step in adaptation to new environments; reducing the cost of directional selection (Ghalambor et al. 2015). The evolutionary response of an individual to selection pressure is dependent on the strength of the directional selection and the amount of genetic variance, allowing plastic individuals to have a higher fitness in the new environment (Gotthard and Berger 2010). Thus, understanding how plasticity in a trait impacts its heritability should allow predicting the rate of evolutionary change in a population (Gibert et al. 2019). The diapause strategy is a good example of adaptive plasticity (Sgrò et al. 2016). However, plasticity can be non-adaptive when the phenotype produced is further away the optimum (sub-optimal) in terms of individual fitness (Whitman and Agrawal 2009; Ghalambor et al. 2015). For instance, extreme environments outside the range of historical conditions may produce non-adaptive plasticity because selection has not had the opportunity to shape the plastic response (Ghalambor et al. 2015). However, it has been observed that "adaptive plasticity" impedes evolution, whereas 'non-adaptive' plasticity facilitates evolution (Ghalambor et al. 2015). Plasticity and bet-hedging strategies are intricately related (Le Lann et al. 2021; Joschinski and Bonte 2021). In the nature, environmental heterogeneity and climatic predictable variability favor the emergence of phenotypic plasticity, which plays an important role in the ecology and evolution of organisms (Fusco and Minelli 2010; Sgrò et al. 2016; Gibert et al. 2019). However, in unpredictable thermal conditions plasticity will not be selected and bet hedging will be favored (Le Lann et al. 2021).

In addition, the effect of environmental conditions on phenotype expression may occur at distinct scales (Rodrigues and Beldade 2020): 1) Modification in progeny phenotype depends on parental environment, 2) modification in adult phenotype in response to adult environment (reversible changes in traits), acclimation correspond a classical example for this response (Sgrò et al. 2016), and 3) change in phenotype that depends on the conditions experienced during development resulting in a phenomenon called developmental plasticity. (Gotthard and Berger 2010). For instance, facultative diapause in insects is a well-documented example of developmental plasticity (Nylin and Gotthard 1998), allowing insects to face with variable environments. Therefore, phenotypic plasticity in a variety of traits will be the first response of insects to environmental changes (Gibert et al. 2019). Understanding the role plasticity and bet-hedging play is important to better know the persistence of individuals and populations in response to changing environments (Gotthard and Berger 2010; Nyamukondiwa et al. 2011; Abram et al. 2017).

## Fitness trade-offs for thermal stress

The expression of most physiological and life-history traits is negatively affected by high levels of thermal stress (see before). According to life-history theory, the fitness of individuals (e.g., reproduction, maintenance, dispersal) are limited by trade-offs that maximize fitness while balancing the energy cost (Stearns 1989), since individuals are unable to optimize the values of all their traits at the same time due to energetic constraints, maximizing the value of one trait (Marshall and Sinclair 2010). More accurately, a trade-off may be defined as a decrease in the investment of non-selected characters that accompanies adaptation to new selective conditions (Pörtner et al. 2006). Life history traits are typically highly sensitive to environmental variability, these thermal sensitivities cause a limit on the ectotherm's performance associated to growth, development, and reproduction (Angilletta et al. 2002). Trade-offs are a main assumption of optimality models of life-history evolution. They offer an explanation for the wide-ranging existence of variable life-history traits in natural populations (Zera and Harshman 2001). In insects, unfavorable environmental conditions can produce important physiological and behavioral changes and, given limited resources, force survival and reproductive trade-offs (Lancaster et al.2017). For instance, it has been observed that starvation act as a positive stressor improving cold tolerance in *Bombyx mori* (Linnaeus) (Lepidoptera: Bombycidae), however at the same time, it acts as a negative stressor and decrease the heat tolerance, which suggest the existence of a trade-off between traits within an individual (Hoffmann et al. 2005). It has been known that one potential cause of trade-offs is the higher allocation of a limited resource: a clear example is that energy allocated to reproduction is unavailable for somatic growth (Angilletta and Dunham 2003). Trade-offs are modulated by environmental context; thus, it is important to consider how environmental change affects trade-off shape (Jessup and Bohannan 2008). Trade-offs between expressed physiological traits can also occur during similar or different times in the life cycle of insects (Zera and Harshman 2001), since this latter is correlated with temperature (Angilletta and Dunham 2003).

Response to stressful temperatures may result in trade-off for a given characteristic related to fitness. It has been proposed that organisms may trade-off thermal tolerance and performance under heterogeneous thermal condition. For instance, it has been observed that repeated freezing events in *D. melanogaster* significantly increased cryoprotection but decreased egg production due to a shift in lipids (Marshall and Sinclair 2018). Therefore, integrating trade-offs is important to understand their response to modifications in seasonal environments (Tougeron et al. 2020). Predicting how often trade-offs limit the evolution of thermal tolerance has strong consequences for understanding species at risk to climate change (van Heerwaarden and Kellermann 2020).

## Seasonal thermal stress

Seasonality describes predictable events referring to both periodic changes in the physical environment and the biological response to these changes (Powell and Logan 2005). Seasonal cycles in photoperiod and temperature are the most important cues used by organisms to maintain internal clock[1]. Generally, the measurement of time has been closely linked to phenology (Powell and Logan 2005; Kellermann and van Heerwaarden 2019) and strongly shapes all aspects of life cycle for ectotherms (Williams et al. 2017). Spatial and temporal variability of temperatures are often considered separately (Roberts 1978). However, in nature, temperatures fluctuate between seasons and varies among habitats (Ragland et al. 2008). In the last fifty years, ecologists have been paying attention to the effects of the abiotic environment on the development, survival and reproduction of insects and how these relationships are modified through time and space (Sinclair et al. 2003).

Changes in seasonal temperatures in insects often require thermal regulations. Therefore, depending on the time of the year, environment can provide resources and favor development or physical conditions are unsustainable and resources are scarce (Danks 2007). The alternation between favorable and unfavorable seasons in temperate regions is the most significant variation in temperatures (Marshall et al. 2020). During the winter season, low surface temperatures are the main feature accompanied by additional abiotic stressors such as wind, precipitation, and solar radiation (Sinclair et al. 2013). In the same way, summer conditions may also be stressful for insects due to the frequency or duration of high temperatures that also induce a decrease of available resources (Andrew et al. 2013). Seasonality tends to translate into seasonal activity patterns in insects. Often, cold seasons alternate with warmer seasons, and rainy periods may alternate with drier times (Wolda 1988). In this context, environmental variation requires conservative physical and physiological

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[1] An “internal-clock” is a central feature of time perception.

adaptations in ectotherms, which determine species distributions and compositions (Marshall et al. 2020).

Insects living in temperate environments have evolved different strategies to cope with the variability of environmental factors such as low winter temperature, summer heat, or low resource availability. These strategies involve behavioral and physiological mechanisms allowing to survive under weather changes and seasonality (Tauber et al. 1986; Tougeron et al. 2020). Below, some of the most relevant mechanisms found in insects are described: 1) Migration, allow insects to connect populations with their habitat requirements through their seasonal movements processes (Bauer and Hoye 2014; Wotton et al. 2019). 2) Diapause, an alternative developmental strategy characterized by developmental arrest initiated in advance of unfavorable seasonal conditions (Tauber et al. 1986; Tougeron 2019) which serve to overcome harsh conditions (Powell and Logan 2005), and 3) ´remain active` that is a physiological mechanism for organisms living in temperate environments with strong seasonality (Powell and Logan 2005). Ectotherms, have direct temperature control (Danks 1987). Thus, an understanding of the physiological traits associated with seasonality is critical for understanding how animals cope with extended periods of stress. On the one hand, it is important to maintain resilience with respect to stochastic climate events and on the other hand, it is advantageous to adapt to changing climate to exploit new environments or persist in the environment of origin (Kurylyk et al. 2014).

## Environmental gradients

An environmental gradient is a gradual change in abiotic factors and, consequently, in biotic factors, through space or time (Barletta and Dantas 2016). The environment is hyperdimensional and consists of all those external variables to the organism. Abiotic factors include temperature, humidity solar radiation; while biotic factors include the existing of all organisms (Hohenegger 2014; Faith and Lyman 2019). Variability in the environment impacts the structure and dynamics of all organisms. These influences from individual to ecosystems level (Figure 1.5) (Danks 1991; Hoffmann and Hercus 2000; Gotthard 2001; Danks 2006). To evaluate the environment variability and its consequences, there are two general assumptions.

First, the latitudinal or/and altitudinal gradient in geographic range size is a consequence of climatic variability and, second, that there is a matching cline in the life-history traits (Gaston et al. 1998).

The latitudinal gradient of the increasing richness of biodiversity from the poles to the equator in many taxa of ectotherms is a recognized pattern in ecology. (Willig et al. 2003; Hillebrand 2004). In the last decades many research also began to evaluate the latitudinal patterns involving ecological functions, evolutionary ecology, physiological and life-history traits, and species interactions (Addo-Bediako et al. 2000; Castañeda et al. 2004; Chown et al. 2004; Frenne et al. 2013; Roslin et al. 2017; Buckley et al. 2017). One of the long-studied patterns is the Rapoport's rule that states that the size of the distribution ranges of species are related to latitude. This rule was later extended to altitude (Rapoport 1975; Stevens 1989; Willig et al. 2003). This rule suggests that more variable climate at high latitudes results in broader physiological tolerances allowing species to inhabit larger ranges (Stevens 1989). The hypothesis of latitudinal biotic interactions predicts that the strength of biotic interactions increases from high to low latitudes (Schemske et al. 2009), suggesting that low levels of competition are due to lower species richness in temperate regions. This hypothesis was initially developed on the striking difference in the diversity and intensity of biotic interactions among tropical and temperate climates (Roslin et al. 2017).

**Figure 1.5.** Effects of environmental variation on individuals, populations and communities

Geographical gradients are characterized by gradual changes although sometimes may be more sudden. (Körner 2007). High latitudes (and altitudes) are often colder than low latitudes (and altitudes) with short growing seasons, conditions that challenge insects to complete their development and to reproduce (Johansson et al. 2020). In the last century, climate change may be the largest anthropogenic disruption on natural systems and its impact on species is likely to vary geographically (Thomas et al. 2004). The high latitudes and high elevations are likely to experience greater warming than the global mean warming, especially in winter (Deutsch et al. 2008). In addition, climate models project that winter and nighttime temperatures (minimum temperatures) continue to increase disproportionately in temperate areas compared to others (Falconnier et al. 2020). These climatic disturbances can shape ecosystems by influencing their species composition, structure and functional processes and the interactions between species and populations (van Baaren et al. 2010; Tylianakis and Binzer 2014; Tseng and Soleimani Pari 2019; Tougeron et al. 2020). Consequently, there is a growing interest in understanding not only the factors of global environmental change and derived disturbances, but also their consequences on the different biological interactions (Rosenblatt and Schmitz 2014). for which a major impact is expected at high latitudes (Beck and Mahony 2018).

Studies comparing populations across sites with contrasting temperatures, such as latitudinal (and altitudinal) gradients, may provide a unique opportunity to investigate how organisms respond to thermal extremes on the structuring and functioning of communities and ecosystems (Addo-Bediako et al. 2000; Sinclair et al. 2003; Chown et al. 2004; Hodkinson 2005; Frenne et al. 2013; Roslin et al. 2017). Therefore, variation of temperature across geographic gradients is expected to produce physiological differentiation and local adaptation of natural populations depending on their thermal tolerances and physiological sensitivities (Roberts 1978; Pörtner et al. 2006; Porcelli et al. 2017), showing that these parameters usually co-vary with geographic clines (e.g., latitude) (Castañeda et al. 2004).

Local adaptation and phenotypic plasticity to thermal environments can cause different patterns of thermal performance across the geographic range of ectothermic species (Yampolsky et al. 2014; Schulte et al. 2014). Whether geographic clines are adaptive depends on the trait and the environmental factors (e.g., temperature). In local adaptation, populations show genetic differences for environmental conditions corresponding to location-specific variations (e.g., local mean, minimum or maximum temperature) (Lancaster et al. 2016; Xue et al. 2019). Local adaptation and phenotypic plasticity may develop together and usually is difficult to separate their relative influences (Lasse Fast et al. 2008). For instance, it has been shown that geographic distribution of *Drosophila* has a strong correlation with its pattern of thermal tolerance. (Xue et al. 2019) and have greater cold tolerance (Schmidt and Paaby 2008). Moreover, because of the stronger influence of season length and temperature on the life cycle of insects, it can be expected a latitudinal variation in diapause expression among populations (Paolucci et al. 2013). In populations of *D. melanogaster* from higher latitudes in USA, it has been observed a greater diapause incidence at high latitudes than low latitudes (Schmidt et al. 2005). Thus, environmental gradients represent a powerful framework for examining the extent to which locally adapted populations vary in their plastic response to environmental variability (De Frenne et al. 2013).

## Strategies employed by ectotherms to escape winter unfavorable conditions

### *Migration*

Under unfavorable conditions, migratory species comprise a significant proportion of all animals (Chapman et al. 2012, 2015). Under both behavioral and ecological definitions of migration, the migration is characterized by some degree of return. (Chapman et al. 2015). The range of movement can vary from within a short distance for some insect species (e.g., wingless aphids) to thousands of kilometers in the case of other species (e.g., butterflies) (Sheikh et al. 2017). The occurrence of a species is typically related to the distribution of resources, but migratory species frequently move across unsuitable habitats while making migratory movements (Flockhart et al. 2015). Therefore, this strategy of the active insects may represent a multigenerational bet-hedging strategy that allows the offspring to avoid deteriorating environmental conditions (Holland et al. 2006). Numerous insect species, of several insect orders migrate poleward from low latitudes each spring to exploit temporary resources where they can reproduce during the summer but are unable to survive over winter (Chapman et al. 2012). Studies on seasonal migration of insects are mostly focused on the monarch butterfly *Danaus plexippus* (Linnaeus) (Lepidoptera, Nymphalidae) (Flockhart et al. 2015) but is also present in other insects (Saunders 2010).

## *Diapause in insects*

Depending on the predictability of temperature and photoperiod changes, dormancy is either quiescent or diapause mediated (Saunders 2020). Quiescence is the response of individual insects to a sudden unanticipated, non-cyclic and usually short duration deviation of normal environmental conditions that triggers the interruption of growth due to environmental conditions. This is probably a phenomenon confined to early winter when periods of adverse conditions (e.g., low temperature) induce a retardation of growth, development, and reproduction (Leather et al. 1995; Hance et al. 2007). The main differences between diapause and quiescence are that in diapause there is a definite preparatory phase (most often initiated by a temperature and photoperiod) which involves metabolic changes (Diniz et al. 2017)

Diapause is a well-studied seasonal survival strategy, where insects respond to environmental cues such as the autumnal changes in day length or night length (i.e., to photoperiod) and decrease in temperature that occur well in advance of approaching winter (Tauber et al. 1986; Tougeron 2019; Saunders 2020) and occurs at a species-specific stage of ontogenesis. Diapause is a dynamic state (Box 2) of low metabolic activity, genetically determined, neuro-hormonally mediated that include several phases. It is characterized by behavioral inactivity, morphogenesis, and reproductive functions arrest, and slowing growth. Contrary, summer diapause commonly occurs in areas with hot and dry summers (the “active” stages of the life cycle are restricted to cooler and wetter seasons) (see below). Once induced, diapause cannot be immediately terminated even if favorable conditions for development appear (Leather et al. 1995; Koštál 2006). On the contrary, in quiescence once the stimulus that induces the process ceases, physiological activity is restored (Diniz et al. 2017).

## Box 2: Diapause phases in insects

Diapause is a dynamic process consisting of three main successive phases (Košťál 2006). These phases are based on physiological processes, which are significantly modified by diverse environmental factors: (1) pre-diapause; (2) diapause; and (3) post-diapause and each phase may include some sub-phases (Figure 1.6). The ***induction*** sub-phase occurs at a genetically predetermined stage of the life cycle and occurs in anticipation of environmental stress. This receptive stage can occur during the life of the individual or in subsequent generations. During this phase, individuals respond to external signals called "token stimuli" (e.g., photoperiod, temperature, etc.) that opens the routes to diapause. These stimuli are either favorable or unfavorable per se, but they are harbingers of impending environmental changes. The ***preparation*** sub-phase generally follows the induction phase. During this phase, insects accumulate and store molecules such as lipids, proteins, and carbohydrates. These molecules are used in the maintenance of the insect during diapause and provide nutrition for the development that follows the termination of diapause. For instance, there may be alterations in the composition of the cuticle due to changes in the composition of carbohydrates and the addition of lipids or decrease in water. These phases are included in the ***pre-diapause*** phase. The ***diapause*** phase begins with the ***initiation*** sub-phase when morphological development stops. In some cases, this change can be very clear and includes the ecdysis going to a specific stage of diapause or it can be accompanied by color changes. There may be enzymatic changes in preparation for resistance to cold, probably decrease of metabolic rate represents the most general feature of this sub-phase. After, the ***maintenance*** sub-phase, the insect's metabolism remains at low rate and developmental arrest persists. In insects that expresses obligate diapause, ***termination*** sub-phase may occur spontaneously, without any external stimulus, in those with facultative diapause a token stimulus is needed for ending diapause. Diapause is terminated by a combination of unknown endogenous processes and specific change of environmental conditions (exogenous diapause), during which insects can respond developmentally to external conditions (Lehmann et al. 2017). Decrease of diapause intensity to its minimum level is promoted by specific changes in environmental conditions (e.g., chilling, freezing) and subsequent resumption of direct development is enabled. Finally, when unfavorable conditions are encountered after diapause termination, most insects remain dormant and enter a state of ***post-diapause*** **quiescence** which allow the organisms to continue development at low rate until reaching positive environmental conditions (Košťál 2006).

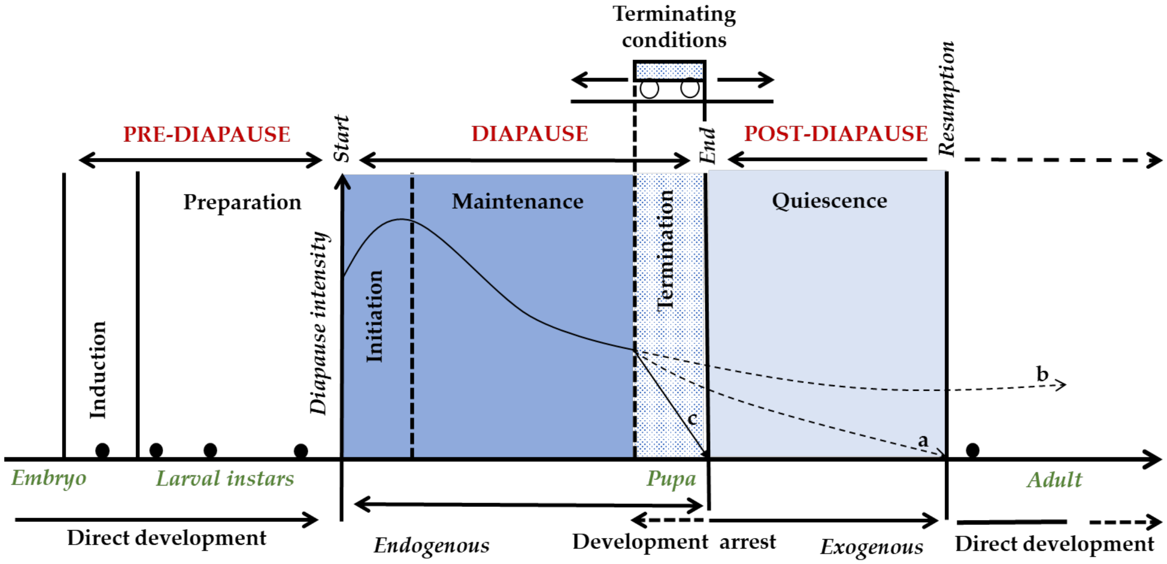


**Figure 1.6.** Description of phases of insects' diapause during the different ontogeny stages, from embryo to adult according to Košťál (2006). Changes in diapause intensity are presented: dotted branches (a and b) apply to the constant conditions, while solid branch (c) applies to the change of environmental conditions.

In diapausing insects, the metabolic rate is held relatively low and constant. The individuals maintain their diapause ensuring that developmental arrest persists for a certain period of time (several weeks or months) (Košťál 2006). Different studies show similarities in specific gene expression between species, proposing the existence of a conserved suite of genes (named "tool-kit") that mediates the behavioral and physiological changes associated with the maintenance of the diapause state across insect species (Poelchau et al. 2013). The term "tool-kit" implies that there is a relatively small set of key regulatory genes for conserved developmental functions (Wilkins 2013). However, there is no consensus about tool-kit conservation across species. Some studies have said that if a diapause tool-kit occurs, it is conserved at the pathway level and not in expression patterns of individual transcripts (Ragland et al. 2008; Des Marteaux et al. 2015; Košťál et al. 2017).

Clock genes are linked to a range of physiological processes including metabolism and hormonal/endocrine signaling, that plays an important role in responding to the diapause inducing environmental signal (Schiesari and O'Connor 2013; Guo et al. 2021). Which hormones are involved depends on the species and the developmental stage in which diapause occurs (Denlinger et al. 2012). Ecdysteroids, juvenile hormones, and the neuropeptides that regulate their synthesis and release, are well-known contributors to diapause regulation. However, newly discovered roles for insulin signaling and additional neuropeptides have expanded our understanding of the regulatory schemes controlling diapause (Denlinger et al. 2012; Schiesari and O'Connor 2013).

Photoperiod in terms of specific effects exerts a powerful influence on insects' response in some basic physiological functions (Saunders 2010). Photoperiod is a reliable indicator of the coming winter season and insects usually use day-length to predict unfavorable conditions (Koštál 2006; Gill et al. 2017; Vázquez et al. 2017). This is the main factor governing diapause induction in many insects (Tauber et al. 1986; Saunders 2020) particularly in species of temperate regions. Diapause induction in some species vary with the interaction of these two factors suggesting that the influence of temperature on diapause induction varies with the photoperiod (and reversely) (Brodeur and McNeil 1989; Bale 2002). Populations of insects of different latitudes respond differently to different photoperiod and temperatures regimes. For instance, populations at northern latitudes of *Nasonia vitripennis* (Hymmenoptera, Pteromalidae) show an earlier switch point and higher proportions of diapausing individuals in longer critical photoperiods. In addition, other environmental stimuli may modulate the diapause expression such as availability of resource and quality (Danks, 1987, Tauber et al., 1998, Saunders, 2014), and intra- and interspecific interactions. Different ways have evolved in insects to use alterations in food quality and quantity (Masaki 1980). Predation is one of the main biotic factors determining individual fitness of the prey. For instance, it has been observed that the production of diapausing eggs by Daphnia magna may be influenced by food concentration under the threat of fish predation (Mirosław and Lusarczyk 2001). Therefore, diapause progression during winter, correct timing of diapause ending is vital for synchronization of emergence with conspecifics and for mobilizing resources when conditions for growth and reproduction become favorable (Lehmann et al. 2017).

### *Summer diapause*

Diapause not only improve survival of insects during winter but may also perform protective and synchronizing functions in summer (Saulich and Musolin 2012). Summer diapause (aestivation) is a phase of dormancy may be defined as diapause induced before the intensity of summer, and followed by reproductive, developmental, or feeding activities in autumn or winter (Masaki 1980). Insects may enter summer diapause to escape from the hot and dry weather and food shortage (Saulich and Musolin 2012). Physiological mechanism is similar to winter diapause and hormonal syndrome associated with summer diapause is quite similar to winter diapause (Masaki 1980; Tauber et al. 1986). For instance, it has been observed that the leaf miner parasitoid *Chrysocharis pubicornis* (Hymenoptera, Eulophidae) expressed diapause with the increase of photoperiod and temperature (Larios et al. 2007), suggesting that summer diapause is induced to overcome harsh thermal conditions. In addition to limited food resources and intra and inter specific competition has been proposed as a factor selecting for diapause (Lalonde 2004). It was observed in aphid parasitoids where summer diapause was induced by female competition in *Aphidius avenae* (Haliday) and *Aphidius rhopalosiphi* (De Stefani-Perez) (Hymenoptera: Braconidae) (Tougeron et al. 2018c).

### *Diapause Cost*

Studies on life history evolution show evidence that winter and summer diapause are costly in terms of fitness (Matsuo 2006). Potential costs of diapause are of various sorts such as metabolic, reproductive, and behavioral ones. When food sources are scarce or unavailable, managing metabolic resources is important during diapause. Insects accumulate reserves prior to diapause, and metabolic depression during diapause promotes reserve conservation (Hahn and Denlinger 2010). Diapausing insects with low energy reserves do indeed have higher mortality during diapause (Hahn and Denlinger 2007). In addition, the energy reserves consumed during diapause have a great effect on post-diapause fitness (Hahn and Denlinger 2007). Prolonged diapause in females of Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera, Chrysomelidae) resulted in a lower offspring survival even though these offspring had faster development times (Margus and Lindström 2019). Also, in the bruchid, *Acanthoscelides pallidipennis* Motschulsky (Coleoptera: Bruchidae), mating success (Burton-Chellew et al. 2007), fecundity and size (Sadakiyo and Ishihara 2012) of adults were reduced when emerging from diapausing pupae compared with the non-diapause individuals. Moreover, it has been observed that an increase in diapause length caused high mortality in diapausing individuals as well as a decrease in egg load, fat reserves and dry weight of emerging adults (Ellers and Van Alphen 2002). In the mosquito *Anopheles gambiae* (Culicidae, Diptera), inhabiting dry savannahs and semi-arid environments, there is a depression in reproduction that is influenced by gonotrophic dissociation over the dry season in diapausing individuals (Yaro et al. 2012; Lehmann et al. 2014). However, individuals that do not enter in diapause die off before or during harsh unfavorable seasons, being extremely costly (Goto and Matsumoto 2018).

### Remain active as a strategy

Low temperatures are among the most important abiotic factors limiting the range of insects (Rivers 2008). In mild environmental conditions, some insect species are known to survive the winter season by maintaining their activity (locomotion, flight, feeding and even reproduction) and entering in short periods of quiescence during the coldest days (Hance et al. 2007). Critical thermal limits (CTLs) in insects' performance at high ($CT_{Max}$) and low ($CT_{Min}$) temperatures are important adaptations that may be shaped by selection to climate variation (Hazell and Bale 2011). However, ectotherms show little variation in $CT_{Max}$ but prominent changes in $CT_{Min}$ (Gabrys et al. 2008). Insects vary considerably in their ability to survive low temperatures (Clark and Worland 2008) where ecophysiological mechanisms have been involved to enhance the capacity of insects to survive low temperatures (Danks 1991; Bale 2002; Denlinger and Lee 2010). Cold hardiness has a highly variable genetic architecture with considerable heritable variation both within and among populations and species (Marshall et al. 2020). This is supported by lower lethal temperatures of winter adult morphs of *Drosophila suzukii* Matsumura (Diptera, Drosophilidae) than summer morphs.

Insects respond to cold environments through a series of physiological adaptations such as freezing tolerance (i.e., insects which have the ability to survive extracellular ice formation) and freezing avoidance by super cooling point (SCP), corresponding to the ability to prevent freezing of the body fluids (Sinclair et al. 2003; Hance et al. 2007). When freezing temperatures occur, insects may survive the formation of ice in their tissues (freeze tolerant), but in general ice formation is lethal (freeze intolerant) (Hance et al. 2007). Proteins also act as antifreeze in insects producing a thermal hysteresis by lowering the freezing point of hemolymph while not affecting the melting point temperature (Addo-Bediako et al. 2000; Block 2003; Sinclair and Marshall 2018). Freeze-avoiding insects have the capacity to reduce the temperature at which their cell fluids crystalize by producing cryoprotectant molecules, removing ice nucleators, synthesizing antifreeze proteins to reduce the nucleation potential of seed crystals, and accumulating sugars and polyols (Bale 1987, 2002; Holmstrup et al. 2002; Sinclair et al. 2003). However, the occurrence of freeze tolerance and freeze avoidance leads to the costs of each strategy (Block 1991).

In addition to affect fitness directly, remaining active as a strategy may produce associated costs (Hance et al. 2007). However, the costs depend on the length and severity of the environmental conditions, since the use of heat tolerance mechanisms have been associated with reduced survival, fecundity, body size, and mating success temperatures (Kristensen et al. 2007). For instance, in many studies focusing on the adult stage, reproductive costs after cold or hot hardening have been confirmed a decreased fecundity (Roux et al. 2010; Zhao et al. 2014; Alemu et al. 2017). In the same way a reduction in fitness-related traits in parasitoids exposed to low temperatures was observed, with an increase in development time and a reduction in the size (Saeed et al. 2021). Then, bet-hedging with individuals staying active and other diapausing can be advantageous especially in the context of climate change with more and more extreme climatic events (Le Lann et al. 2021).

## Overwintering strategies: the case of aphid parasitoids

Parasitoids are insects that live in close relationships with their host, whose adult females lay their eggs inside or on the host. The parasitoid larvae develop by feeding on the host bodies, resulting in the death of the host (Godfray 1994). Insect parasitoids are important components of terrestrial ecosystems in terms of biodiversity, abundance, and ecological functions, whose life histories are closely linked to those of their hosts (Godfray 1994; Quicke 2012). Parasitoids play a crucial role in regulating population dynamics of their hosts in both natural and managed environments (Leblanc and Brodeur 2018; Cloyd 2020). The effect of environmental conditions such as temperature and photoperiod on these groups is of particular interest since these factors may disrupt the synchronization of their life cycles (Tougeron et al.

2020). Aphid hosts and parasitoids may respond differently to changes in the mean temperature due to trade-offs, evolutionary history and genetic background (Le Lann et al. 2021). Aphid parasitoids have several adaptations to synchronize their lifecycle with their aphid hosts (Langer and Hance 2000). Being ectotherms, parasitoids are expected to be sensitive to environmental variability both directly due to changes in abiotic conditions but also to biotic conditions due to their host (Polgár et al. 1995, Polgár & Hardie 2000, Tougeron et al. 2020a), and indirectly due to changes in the individual, population and community levels of lower trophic levels (Tylianakis et al. 2007; Tougeron et al. 2018b; van Baaren et al. 2020). Winter conditions are both a selective pressure and a modulator of the overwintering strategies in aphid parasitoids (Andrade et al. 2016; Tougeron et al. 2017, 2018b).

### *Diapause in aphid parasitoids*

A review by Polgar and Hardie (2000) showed that diapause induction appears to be genetically determined in univoltine parasitoids independent of abiotic factors. In contrast, multivoltine parasitoids have species-specific regulatory systems and they seem to be able to respond to a variety of cyclical changing environmental abiotic factors like photoperiod and temperature and most of the aphids parasitoids (Braconidae, Braconidae) group are multivoltine species (Polgár and Hardie 2000). In addition, changes in host quality (i.e., species, size, morph), maternal effects including intra/inter specific competition may regulate parasitoid diapause (Brodeur and McNeil 1989; Langer and Hance 2000; Tougeron 2019; Tougeron et al. 2020). As any other insects, parasitoids need to accurately detect and use indicative cues of environmental variability (Le Lann et al. 2021). Commonly, in aphid parasitoids species, diapause is facultative and only a part of the population enters in diapause (Polgár & Hardie, 2000), in which specific developmental stages are sensitive to environmental stimuli (Tougeron, 2019). Diapause occurs in a genetically pre-determined stage of the insect life cycle (Koštál 2006; Tougeron et al. 2017) and it has been observed that cues acting on diapause initiation in aphid parasitoids act during embryonic and first-instar larval development (Brodeur and McNeil 1989). These species overwinter as a diapausing final-stage larva within the mummified host (Christiansen-Weniger and Hardie 1999) (Figure 1.7). Therefore, diapause expression may be measured as the proportion of individuals entering diapause at this specific state of the total number of individuals evaluated (Valera et al. 2006; He et al. 2010).

**Figure 1. 7.** Golden-yellow prepupae diapausing of *Aphidius ervi,* 36 days following mummification.

In different species of *Aphidius* for instance, temperature and photoperiod, as well as the interaction between these two factors are environmental stimuli that could trigger diapause initiation. In *Aphidius nigripes* parasitizing *Macrosiphum euphorbiae* (Hemiptera, Aphididae), the diapause reaches an incidence of 99% under low-temperature and short photoperiod (Brodeur and McNeil 1989). In the same way, diapause incidence above of 65% was recorded in similar conditions in *A. ervi* and *A. rhopalosiphi* showing that the diapause induction increases survival in winter in these species (Langer and Hance 2000). In some aphid parasitoids the percentage of individuals entering in diapause decreased with the increase in temperature as well as their aphid hosts as consequence of winter warming (Tougeron et al. 2017). Diapause helps maintaining the synchronization between hosts and parasitoids (developmental or phenological) (Polgár et al. 1995; Langer and Hance 2000) even though, pest and natural enemies may have different thermal preferences or different capacities to survive to extreme temperatures (Hance et al. 2007).

For parasitoids, in addition to environmental conditions, the quality of their environment depend on the suitability and abundance of hosts which vary throughout the seasons (Vercken et al. 2015), where maternal perception could contribute to diapause induction (Tougeron et al. 2020). Therefore, diapause expression may be sensitive to any modification in seasonal host densities and food shortage (Saunders 1966; Polgár et al. 1991). For instance, it has been shown that diapause incidence is higher when *A. nigripes* parasitizes smaller hosts (Brodeur and McNeil 1989). In addition, the parasitoid *A. avenae* have developed behavioral adaptations to respond to intra or interspecific competition (Tougeron et al. 2018c). Diapause levels and development times increase over generations trough transgenerational effects (Tougeron et al. 2020). Finally, for diapause termination, temperature is the main determinant directly on the parasitoid or indirectly through the aphid host (Tougeron et al. 2020).

Entering, maintaining, and terminating diapause may alter different aspects of the insect physiology since it is not reversible and involving cost-benefit balance and evolutionary trade-offs (Bale and Hayward 2010; Tougeron et al. 2020). However, there are few studies on the consequences of diapause on life history traits in aphid parasitoids. Longevity is often reduced in insects, including parasitoids, emerging from diapause due to physiological stress. For instance, diapausing *Praon volucre* have a reduced longevity and a negative relationship exists between the time to emergence and longevity in diapausing wasps (Colinet et al. 2010). In this case a higher lipid mass were present in diapausing mummies compared than non-diapausing ones. Reserves of lipids might improve survival during a long period of diapause but also as energy resources for eggs production after diapause (Colinet et al. 2010). Also, population sex ratio emerged after diapause was male biased (Christiansen-Weniger and Hardie 1999).

### *Remain active as strategy in aphid parasitoids*

In winters characterized by mild temperatures, the absence of lethal frosts allows aphids and their parasitoids to remain active (Tougeron et al. 2018b). Anholocyclic aphids (permanently parthenogenetic) or monoecious (non-host-alternating) (Bell et al. 2015) are well known to adopt this strategy. Polgár et al. (1995) noted that parasitoids linked to anholocyclic aphids in areas with mild winters could present a plastic response to diapause induction, because these aphids may provide an alternative resource to enhance early parasitoid activity in winter. Anholocyclic cereal aphids under mild winters offer a new resource to the parasitoids throughout the seasons (Andrade et al. 2015). For parasitoids, the quality of their environment depends on both the suitability and abundance of hosts. Parasitoids that do not enter in diapause should be more cold-resistant to low temperatures during immature and adult stages (Tougeron et al. 2018a). Therefore, overwintering as an active adult in parasitoids may consequently become a more common strategy, suggesting that physiological and life-history traits can adjust rapidly through developmental thermal acclimation (Rigaux et al. 2000; Levie et al. 2005). For instance, cold stress as factor of selection in *Aphidius avenae* (=picipes) (Haliday) result in rapid adaptive to such stress (Amice et al. 2008). In *Aphidius colemani*, the quantity of fat available for potential emerging adults declined with duration of cold exposure (Colinet et al. 2006) and this may decrease their adult longevity.

## Biological control context

Climate change is a change in the long-term weather patterns that characterize the different regions of the world. Global average temperatures have increased by about 1 °C since the pre-industrial era and that anthropogenic warming is adding around 0.2 °C to global average temperatures every decade and may facilitate alien species invasions, causing greater damages to natural ecosystems and agroecosystems (Thomas et al. 2004; Pecl et al. 2017; Marshall et al. 2020). These seasonal and long-term changes would affect population dynamics of insect pests and the activity and abundance of natural enemies (Furlong and Zalucki 2017)

### How climate change affects biological control?

Biological control is a key ecosystem service that underpins sustainable approaches to their pest management, thereby providing significant environmental benefits. It has been known that the success of the biological control depends on the ability of natural enemies to become established in their new environment (Henry et al. 2010; He et al. 2010; Shields et al. 2019). In parasitoids, changes in the seasonality may have consequences on host–parasitoid synchrony, food-web interaction, and ecosystem services such as biological pest control (Tougeron et al. 2020). In fact, desynchronization in host-parasitoid phenology may induce changes in parasitoid diapause expression (Tougeron et al 2020). It is known that the optimal temperature (Topt) for the development of natural enemies is significantly lower than that for their hosts (Furlong and Zalucki 2017), for this reason, climate change conditions would promote the development of more generations per year in insect pests and facilitate their dispersion (Thomson et al. 2010).

In the last years, there is much interest that climate change will affect ecological networks to become rewired because of the inability of heat-sensitive species to tolerate or adapt to changing climate (Schmitz and Barton 2014). In this context these inconsistencies could contribute to asymmetrical outcomes in host–parasitoid interactions, resulting in reduced parasitoid efficacy and the possible exclusion or addition of parasitoids (Furlong and Zalucki 2017). It has been known that variations in host–parasitoid food-web composition may decrease the effectiveness of biological control through increasing intraguild competition for the addition of new antagonist species (Tylianakis and Binzer 2014; Tougeron et al. 2020). Changes in aphid–parasitoid synchrony may have detrimental effects on biological control (Andrade et al. 2016; Tougeron et al. 2017, 2020). If parasitoids emerge earlier than their hosts because their development shows a sharper response to temperature, it could lead to the eventual extinction of the parasitoid population (Furlong and Zalucki 2017; Zhang et al. 2017). Contrary, it is possible that potential aphid-parasitoid desynchronization does not have negative effects on biological control, since functional redundancy within a guild of parasitoids may control the aphid pest population (Tougeron et al. 2017; Feit et al. 2019).

In addition, climate change can alter plant composition such as secondary metabolites (Zvereva and Kozlov, 2006). These modifications may change pest feeding behavior and the nutrient content of the pest, which can affect the parasitism efficiency (Ode et al., 2014). For instance, if plant quality reduces host size, parasitoids developing inside of smaller hosts may produce smaller parasitoids at emergence (Kouamé and Mackauer 1991), affecting their fitness, since smaller adult parasitoids have lower fecundity (Godfray 1994), reducing the efficacy of biological control. Therefore, predicting how climate change will affect natural enemies and pest represents an important research challenge (Selvaraj et al. 2013; Thurman et al. 2017).

# Biological model

## Aphids

Aphids include a large group of insects in the superfamily Aphidoidea (Hemiptera) that are phloem-feeding insects. In the world, it has been recorded more than 4000 aphid species, of which around 250 are among the most destructive pests causing serious damages in almost all agroecosystems worldwide, by decreasing the vigor of cultivated plants directly or acting as vectors of plant viruses causing diseases (Blackman and Eastop 2000; Schmidtberg and Vilcinskas 2016; Hardie 2017). One of the most relevant characteristics of aphids is that they show a variety of polyphenisms in response to environmental conditions (biotic and abiotic), including winged and/or wingless and sexual and/or asexual individuals in the same population (Figure 1.8A) (Simon et al. 2002). Usually, aphids reproduce by combining sexual and asexual phases (holocyclic), alternating between primary winter host and secondary summer hosts (Figure 1.8B). Also in most aphids, cyclical parthenogens can produce cold-resistant diapausing eggs, providing a major short-term ecological advantage of sex (Simon et al. 2002). However, under specific conditions, where the primary host is scarce and winters are mild, aphids are completely parthenogenetic (anholocyclic). Continuous asexual reproduction occurs in the autumn and winter with the winged females colonizing available host plants, then moving to the same host or others during the spring and summer (Figure 1.8C) (Finlay and Luck 2011; Hardie 2017). In general, aphid species attacking cereal crops in Chile are characterized by very low levels of genotypic and genetic diversity, suggesting that obligate parthenogenesis is the primary reproductive mode (Figueroa et al. 2018). The availability of host plants and their phenotypic plasticity that increases the chance of aphids to invade new environments, are among the main variables supporting their success as an invasive pest in new geographic areas (Figueroa et al. 2018; Rubio-Meléndez et al. 2019). They represent an important resource for their natural enemies which include hover flies, lady beetles, lacewings, Carabid beetles and spiders (Zhao et al. 2015) and their main parasitoids belong to the Aphidiinae group (Hymenoptera, Braconidae) (Starý 1995).

**A. Parthenogenetic *Sitobion avenae***

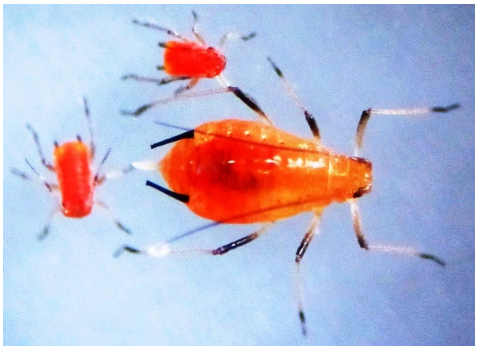
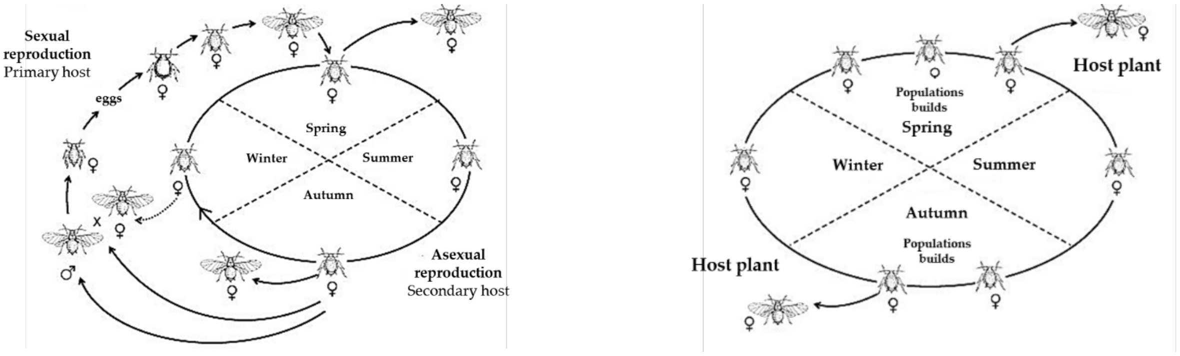


**Figure 1.8.** (A) Parthenogenetic grain aphid, *Sitobion avenae*, a cosmopolitan species on grasses and cereals. (B) Typical holocyclic life cycle of aphids. The species alternates between the primary winter host, and secondary summer hosts. Anholocyclic life cycle of aphids. Continuous asexual reproduction occurs with the winged forms moving from different host plants. Modified of Finlay & Luck (2011).

**B. Holocyclic life cycle** **C. Anholocyclic life cycle**



## Aphid parasitoids

Aphid parasitic wasps are important natural enemies that help to control pest aphid populations in different crops. They are grouped in two taxa: (1) the sub-family Aphidiinae (Hymenoptera: Braconidae) and (2) the genus *Aphelinus* (Hymenoptera: Aphelinidae). These taxa are specialized attacking aphids and lay their eggs in larvae and adult instars of their host (Figure 1.9A) (Le Ralec et al. 2010). Aphid parasitoids are solitary koinobiont parasitoids, i.e., parasitic larval stage that develops by feeding on a single host resulting in the death of its host and the resulting adult parasitoid is a free-living insect (Mills 2009) (Figure 1.9B).

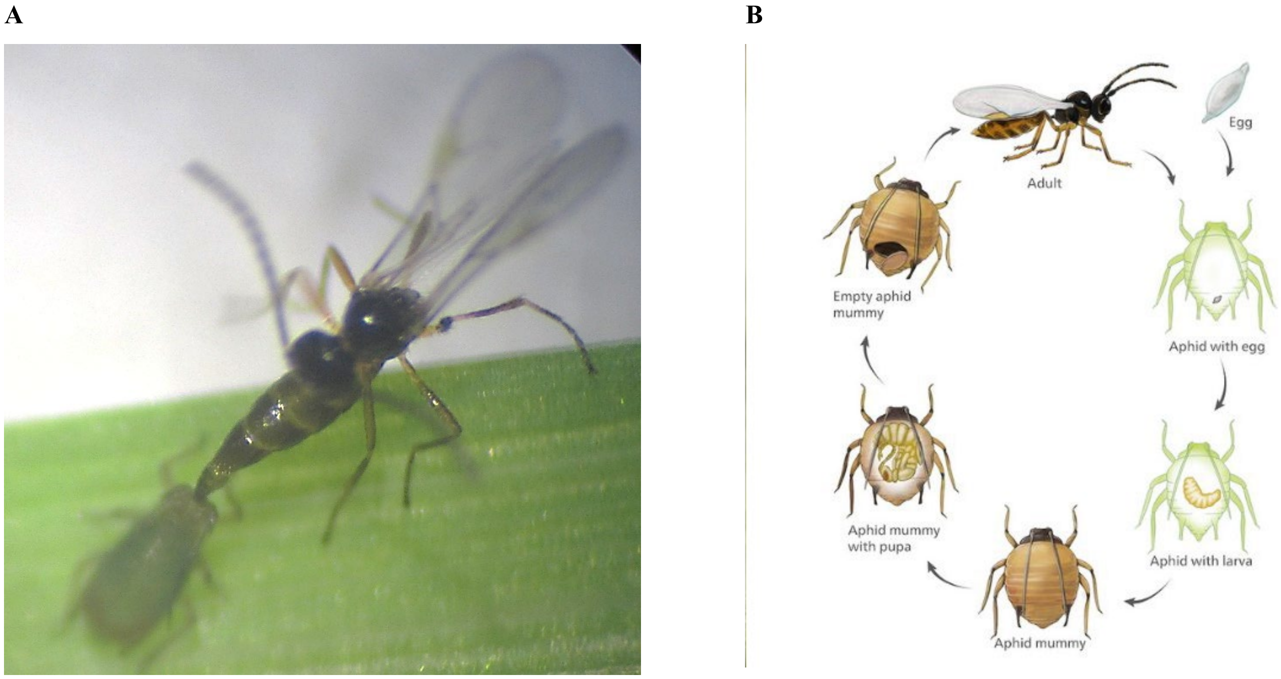


**Figure 1.9**. **(A)** *Aphidius platensis* ovipositing on *Rhopalosiphum padi* **(B)** Biological cycle of an aphid parasitoid (https://twitter.com/KoppertCa)

The life histories of parasitoids are closely linked to those of their host (Godfray 1994). Parasitoids respond to different stimulus to locate and recognize their hosts (Rehman and Powell 2010). In host-parasitoid interactions, an important step for the parasitoid females is to locate and select suitable hosts to optimize their fitness. Host preference determines host acceptance or rejection, and therefore affects their oviposition behavior (Henry et al. 2010; Zepeda-Paulo et al. 2013).. During immature development, the aphid represents the whole nutritional and physiological environment for parasitoids (Godfray 1994; Henry et al. 2010). Fitness of parasitoids may be estimated by measuring different life-history traits such as size, survival, development time, sex ratio, fecundity for females and mating ability for males, although these proxies are difficult to assess under natural conditions (Godfray 1994; Gibert et al. 2019).

The ability of a parasitoid to remain active under extreme temperatures is an important part of its fitness (Le Lann et al. 2021). Variation in temperature above and below the parasitoid's survival threshold results in adverse effects on physiological responses (Hance et al. 2007). For instance, in aphid parasitoid used as models in this study, the lower developmental thresholds for *A. colemani* a close species related to *A. platensis* (Tomanović et al. 2014) were estimated to be 2.9 °C (Zamani et al. 2007), while the temperature thresholds for *A. ervi* was estimated as 2.2 ◦C for egg-mummy development and 6.6 ◦C for mummy-adult development (Sigsgaard 2000). As observed by Colinet et al. (2007) and Sigsgaard (2000a), *A. colemani* and *A. ervi* showed a negative correlation with temperature during development suggesting that the developmental time of parasitoids is also temperature-dependent like with other ectotherms (Zuo et al. 2012). In addition, the seasonal activities of some parasitoid species seem to match their thermal tolerances (Le Lann et al. 2011). The resistant individuals may perform almost as well as those developed in optimal temperatures (Ismail et al. 2013), but show a reduction in their size showing that fluctuating temperatures induced less damage than the constant extreme low ones in *A. ervi* (Ismail et al. 2012). However, in *A. colemani* the sex ratio after cold exposure indicates that males may be more susceptible to cold injury than females (Colinet et al. 2007).

On the other hand, in the aphid parasitoid *A. ervi* from mild winter areas the incidence of diapause expresses very low levels (between 5 and 10%) when exposed to short photoperiod and low temperature conditions (Tougeron et al. 2017; Saeed et al. 2021). On contrary, populations from colder areas express high diapause levels (around 90%) (Tougeron et al. 2018d). While for *A. platensis* there are no known studies in this regard. However, it has been recorded that *A. colemani* did not express winter diapause when exposed to similar conditions (Benelli et al. 2014). Therefore, studying the thermal limits is therefore one of the ways to understand how extreme climatic events could affect the fitness of parasitoids.

## *Chile as study case:*

The Chilean central-south valley compromises a mosaic of agricultural, forest and natural habitats in a climatic gradient from the IV to the X region (Latitude 29° - 42° S) and is characterized by arable fields typically annual crops (at the time that this work was carried out, 2016/17/18 season). Approximately, 39% of cereal sowings are carried out in the La Araucanía region, 24% in Biobío region and 14% in Maule region. The main cereal crops are in an extension of 395,556 ha which includes wheat *Triticum aestivum* (56.89%), oat *Avena sativa* (34.59%), barley *Hordeum vulgare* (3.43%) and triticale that is a hybrid of wheat (*Triticum*) and rye (*Secale cereale*) (5.09%) among Coquimbo region and Los Lagos region (Figure 1.10A) (ODEPA 2019). Gradient should include latitudinal variation (photoperiod, temperature and radiation variation) and altitudinal (for below zero days variation-frost, etc.). In Chile, historical temperature shows a descending trend over the ocean and on the coast, while in the central valley the trend has been rising, especially near and in the mountains (Falvey and Garreaud 2009). Chile's large latitudinal extent led to a variety of climatic gradient. According to Mutz et al.(2021), the Niño Southern Oscillation, south Pacific Anticyclone and mid-latitude westerlies are important drivers on climate and weather in Chile, having a result of a large temperature gradient (Figure 1.10B).

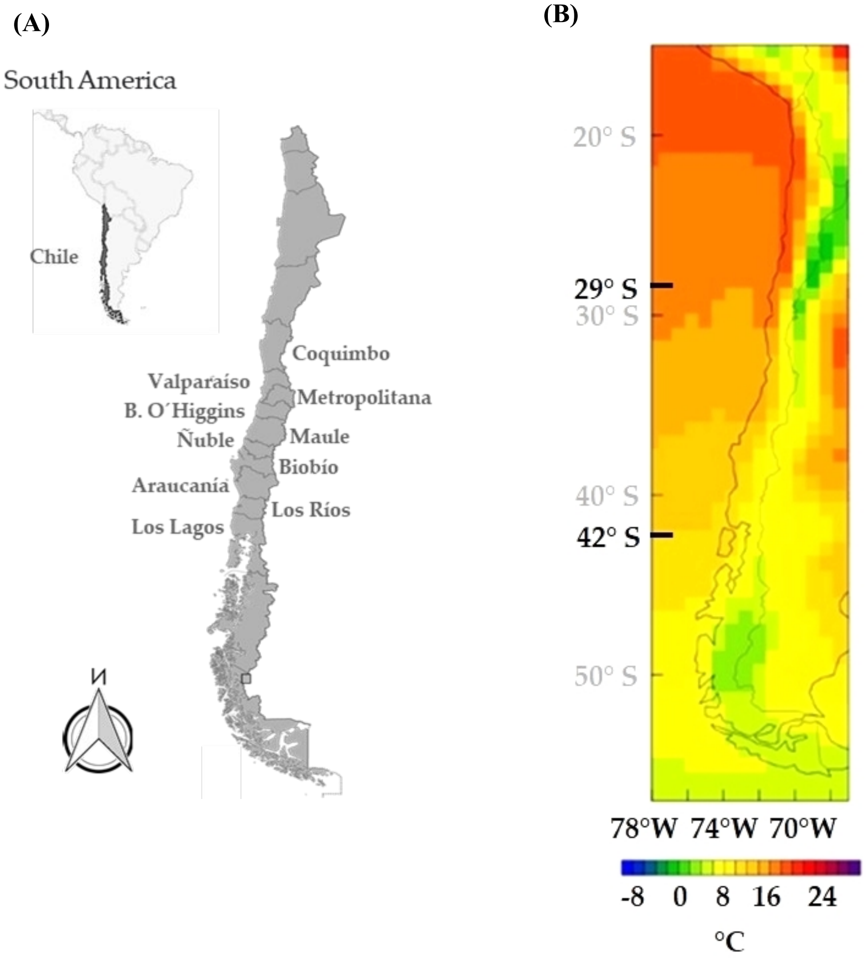


**Figure 1.10**. (A) Map of Chile showing the regions corresponding to the central- south valley (Modified from https://es.wikipedia.org/wiki/Archivo:Mapa_de_Chile_(regiones).svg). (B) Mean annual temperature near surface (2 m) highlight the pronounced climate gradients along latitudinal gradient in Chile (Mutz et al 2021).

The central-south valley of Chile is a region where the cereal aphids and their parasitoids have different origins (Starý et al. 1993). The first reports of *Rhopalosiphum padi*, *Sitobion avenae*, *Metopolophium. dirhodum* and *Schizaphis graminum* (the main pests on cereal crops (Nieto Nafría et al. 2016; Ortiz-Martínez and Lavandero 2018)) presence in Chile were made in the 1960-1970s (Zuñiga 1986). Their populations are characterized by low genetic variability and are predominated by few ´superclones’ with low genotypic diversity, suggesting that obligate parthenogenesis is the primary reproductive mode (Figueroa et al. 2018). Natural enemies associated with these aphids include parasitoid species of the Aphidiinae group (Hymenoptera: Braconidae), many of which were introduced from Europe (Zuñiga et al. 1986; Starý 1995) approximately 50 years ago as part of a classical biological control program (Zuñiga et al. 1986). These species are represented mainly by the genus *Aphidius*, including *A. ervi*, *A. uzbekistanicus*, *A. rhopalosiphi*, *A. avenae, A. platensis*, and *A. matricariae*, although *Praon volucre*, *P. gallicum* and *Lysiphlebus testaceipes* were also recorded. Furthermore, *S. avenae* and *R. padi* appeared as the common resource for the same parasitoid assemblage (Zepeda-Paulo et al. 2013). Therefore, the study of aphid-parasitoid interactions are suitable biological models to explore ecological and evolutionary theories (Le Ralec et al. 2010) along a climatic gradient.

## Research questions and hypotheses

In this research, we will tackle the following questions:

- How do the composition and abundance of aphids and their parasitoids vary along the latitudinal gradient in Chile?
- How is diapause expression partitioned among and within aphid parasitoid guild populations?
- If Chilean aphid parasitoid express diapause, how plastic is this trait?
- What are the main stimuli for diapause induction?

  What are the ecological consequences of abiotic and biotic environmental changes for aphid parasitoid fitness, population dynamics and community functioning?

Our general hypothesis is that parasitoids face different environmental conditions (abiotic and biotic) along a latitudinal gradient, that modify their overwintering strategies. On one hand, it is expected that parasitoids are more active in warmer than colder latitudes, therefore parasitoids may respond in a plastic way to environmental conditions through their physiological and life-history traits. On the other hand, harsher winter conditions may induce more diapause, and populations originating from cold winter areas will show a higher diapause incidence. We predict that diapause incidence on each species varies along the latitudinal gradient with more diapause incidence in coldest areas, influencing in turn aphid-parasitoids interactions. Therefore, a decrease of parasitoid activity during winter in the coldest areas should be observed.

The diapause incidence of aphid parasitoid species will differentially affect the relative abundances of the different parasitoid species in a latitudinal gradient, affecting the composition of the natural enemy assemblages and their interactions with their aphid hosts:

- The winter composition of the parasitoid and aphid community differs according to temperature conditions encountered along the latitudinal gradient.

Abiotic and biotic conditions affect differentially the diapause incidence in aphid parasitoids:

- Winter conditions of temperature and photoperiod will have an effect on diapause, thus, the incidence of parasitoid diapause is higher at lower temperatures and photoperiods
- Diapause expression is sensitive to any modification in seasonal host densities, food shortage and the maternal effects experienced by the mother, so, diapause incidence is higher when females encounter low aphid densities and when females experience high levels of interspecific competition Therefore, to synchronize with the life cycle of their hosts, parasitoids should thus enter in diapause if few hosts are available and suitable.

## Thesis organization

**Part 1.** In the **Part 1** of the research, **Chapter I**: General introduction, the background and state of the art are presented covering the topics of Environmental gradients, thermal tolerance, plasticity, dormancy in insect and overwintering strategies in aphid parasitoids. We detail the mechanisms by which latitudinal gradient may affect their overwintering strategies. In addition, the biological model of study and research questions and hypotheses are included.

**Part 2:** In this part, we are interested in describing the structure of aphid-parasitoid assemblages in the central-south valley of Chile in winter**.**

- **Chapter 2 (*Composition and structure of winter aphid–parasitoid food webs along a latitudinal gradient in Chile*).** We test how the composition and food-webs may differ among different locations on a given latitudinal gradient. In this chapter, we evaluated the variation in species number and interactions within the guild of cereal aphids and their primary parasitoids and we include the secondary parasitoids along a latitudinal gradient during winter. Aphid hosts and their parasitoids may also respond to temperature variations that exist along a latitudinal gradient, therefore

  We tested the following hypotheses: (1) The number of species expressing diapause and the rate of diapause within a population is higher in cold than warm areas, in turn influencing interactions within food webs; (2) the diversity and abundance of aphid and parasitoid species that are active during the winter increases in warmer areas; (3) a decrease in parasitoid activity during the winter in the coldest areas decreases the diversity of interactions (Shannon diversity of interactions index), affecting the proportion of possible links for each host–parasitoid pair species (connectance) and the symmetry of the network (web asymmetry). Likewise, declines in the weighted mean number of aphids used by each parasitoid species (generality) and the mean number of parasitoid species attacking each aphid species (vulnerability) should be observed. Finally, since there is greater stability and less variation in the species composition in warm areas, the level of specialization (H2) should be greater in cold areas.

**Part 3:** We explore in the lab the overwintering strategies and life-history traits of several populations of parasitoids species collected along a latitudinal gradient in Chile. In addition, we evaluated the biotic and abiotic factors affecting the diapause incidence in parasitoids.

- **Chapter 3 (*Overwintering strategies and life-history traits of different populations of Aphidius platensis along a latitudinal gradient in Chile*).** We assessed the effect of photoperiod and temperature on overwintering strategies of the main aphid parasitoid and measure the rate of diapause according to the origin of the populations. For the individuals that do not enter in diapause, we evaluate their physiological and life-history traits along a latitudinal gradient in link with their physiological thermotolerance. We used five populations of *Aphidius platensis*, the most abundant species in winter, sampled along a latitudinal gradient. In addition, the winter temperature variation along the latitudinal gradient was analysed.

The following hypotheses were tested: (1) *A. platensis expresses* low diapause levels, (2) if diapause is expressed, then its level should be higher in parasitoid populations from cold localities than from mild ones, (3) for individuals that remain active during the winter, developmental time should be longer and with larger body size in populations from cold latitudes according to the temperature-size rule, (4) adults from cold latitudes should be more cold-resistant than those from mild environments, affecting numerous other life-history traits, (5) since mass/size are positively correlated with fat reserve content and other life history traits, we expect that individuals from colder latitudes that develop in winter conditions, will have a higher fat content, and (6), they should display higher egg-load and produce larger eggs.

- **Chapter 4 (*Biotic and abiotic factors inducing winter and summer diapause in Aphidius ervi*).** In this chapter, the winter and summer diapause incidence was explored on Chilean populations of *Aphidius ervi* sampled across a latitudinal gradient on cereal fields. In addition, of abiotic conditions, we tested the effect of aphid host species, host density and of maternal competition on winter and summer diapause. We tested the following hypotheses: (1) Since there is no clear latitudinal gradient in temperature in the south-central valley of Chile, the frequency of diapause should be similar along the latitudinal gradient (2) The incidence of winter diapause in parasitoid offspring will be higher on *A. pisum* than on *S. avenae*. Besides, the level of diapause could be linked to the scarcity of the resources, which can also be experienced when host density decreases or when competition among parasitoids increases, thus: (3) When females face low aphid densities, the diapause incidence may increase in winter and summer conditions, and 4) The incidence of diapause may be higher when females experience an increase in competition levels in winter and summer conditions.

**Part 4:** In the **Chapter V**, the general discussion shows the main findings, connections among the specific chapters and reflections to the aims and hypotheses. Perspectives gives the future research insights of the research field, and we give the general conclusions.

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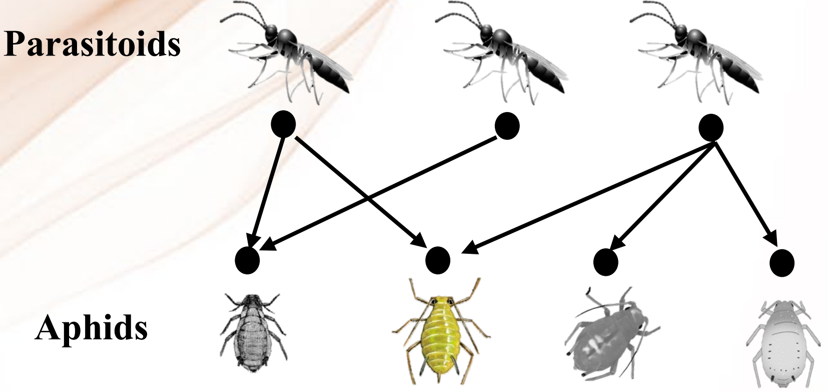
# Part 2

## Aphid-parasitoids food webs composition



## Chapter II:

## Winter aphid–parasitoid food webs

# Composition and structure of winter aphid–parasitoid food webs along a latitudinal gradient in Chile

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## Abstract

All species interact in complex antagonistic or mutualistic networks that may be driven by turnover in species composition due to spatiotemporal environmental filtering. Therefore, studying differences in insect communities along environmental gradients may improve our understanding of the abiotic and biotic factors that shape the structure of trophic networks. Parasitoids are interesting models to do so, due to their intimate eco-evolutionary relationship with their hosts. We explored the differences in cereal aphid-parasitoid-hyperparasitoid food webs in winter among nine localities in Chilean central-south valley, on a gradient of 1200 km from north (29° S) to south (40° S). We hypothesized that diapause incidence increases in the coldest areas, resulting in a lower number of parasitoid species active during the winter. Consequently, network specialization, generality, and vulnerability indexes should increase with decreasing latitude, which implies fewer and more weakly connected links per parasitoid species, through an increased fraction of basal host species. Based on the severity of winter, three areas along the explored gradient were distinguished, but clustering did not follow a clear north-south latitudinal gradient. However, few differences were observed in overwintering strategies, with very low levels of diapause in all localities, and no major differences in food-web composition. The major differences along the gradient were the relative abundances of the different aphid, parasitoid and hyperparasitoid species, with more spatial and temporal variations observed in the less abundant species. Our results provide a better understanding of the activity and abundance of aphid parasitoids during winter in relation to climatic conditions.

**Key words:** latitudinal gradient; community; diapause; host-parasitoid interaction; network structure

# Introduction

One of the most important challenges faced by ecologists is determining the roles of latitudinal and altitudinal environmental gradients in structuring organism communities (Schemske et al. 2009; Dyer and Coley 2009). Areas with different environmental conditions provide an exceptional natural laboratory with which to investigate the roles of abiotic variations on the structure and function of ecosystems (Ibáñez et al. 2007; Frenne et al. 2013). Some species are widely distributed along geographic gradients, so populations living at the upper and lower geographic extremes experience quite different environmental conditions, especially with respect to the local climate (Hodkinson 2005). The thermal environment is characterized by colder conditions (Wielgolaski and Inouye 2003) at high latitudes and altitudes, with temperature being the most important abiotic factor that constrains the abundance and distribution of poikilothermic animals (Bale 2002), affecting their individual performance, ecological interactions, community composition (Bale 2002; Williams et al. 2015) and food-web stability (Gibert 2019).

Species are linked together in networks of ecological interactions (e.g., mutualistic or antagonistic) that contribute shaping the community structure (Paine 1988; Tylianakis and Morris 2017), and these networks change according to the turnover in species composition, which results from spatiotemporal environmental filtering (Pellissier et al. 2018). Therefore, studying the differences in communities along environmental gradients may improve our understanding of the factors that shape the network structure. Organisms distributed along environmental gradients respond to both direct variations in abiotic factors (Van Veen et al. 2006; Tylianakis and Morris 2017; Zhang et al. 2017) and indirect variations through the responses of other species they interact with (Hodkinson 2005; Ings et al. 2009). A decrease in species richness at higher latitudes and altitudes (i.e., in coldest environments) is commonly observed (Lawton 1999; Hillebrand 2004), and communities in these environments are often less complex with fewer trophic levels and smaller interaction ranges (Vázquez and Stevens 2004; Neutel et al. 2007; Schemske et al. 2009; van Baaren et al. 2020), which are important determinants of the food-web structure (Stevens 1989; Van Veen 2009).

In temperate regions, winter conditions are among the major factors limiting the species distribution area and activity window (Bale and Hayward 2010; Kong et al. 2019). For example, food web structure simplification has been observed during winter, suggesting that community stability is vulnerable to abiotic changes that occur during this season (Peralta-Maraver et al. 2017). Three different strategies have been developed by insect species living in temperate areas to allow them to survive winter: migration, remaining active, or entering diapause (i.e., developmental arrest) (Bale 2002; Hance et al. 2007; Bale and Hayward 2010). The relative use of these two last strategies leads to variations in the proportion of individuals/species that remain active or enter diapause (Paolucci et al. 2013) along geographic gradients, therefore affecting population dynamics and the food-web structure (Dyer and Coley 2009; Petermann et al. 2015; Pellissier et al. 2018).

Host–parasitoid interactions are a good model that can be used to understand how environmental gradients affect the food-web composition and structure (Stireman 2016). Network metrics have been extensively used to evaluate the structures of ecological communities (Van Veen et al. 2006; Tylianakis et al. 2007, 2010). Here, we used six of these quantitative metrics to understand the effects of an environmental gradient on the food-web structure. The interaction-Shannon's diversity index (i.e., network entries), the realized proportion of potential links between host and parasitoid species (i.e., connectance), the weighted mean of host species per parasitoid species (i.e., generality), the weighted mean number of parasitoid consumers per host species (i.e., resource vulnerability), the balance between the numbers of host and parasitoid species (i.e. web asymmetry), and the level of specialization (i.e., H2) were assessed (Bersier et al. 2002; Bascompte et al. 2006; Van Veen 2009). These metrics can be used to disentangle important patterns of how ecological communities change in response to environmental factors, including host–parasitoid interactions (Tylianakis et al. 2007; Tylianakis and Binzer 2014; Tougeron et al. 2018a). They are often correlated but allow the assessment of slightly different web properties (Maunsell et al. 2015) and are an appropriate way to elucidate general trends in the food-web structure (Banašek-Richter et al. 2004).

Aphid hosts and their parasitoids may also respond to temperature variations that exist along a latitudinal gradient (Gibert 2019). Differences in the niche breadth among species inhabiting cold and warmer environments could lead to differences in the number of interactions that occur (Vázquez and Stevens 2004; Cirtwill et al. 2015), whereby aphid–parasitoid communities have greater stability and less variation in warmer areas than in colder ones (Maunsell et al. 2015; Morris et al. 2015). Therefore, the level of specialization decreases in colder areas due to a shortage of available hosts, leading to a high level of competition (Andrade et al. 2016). In addition, in the context of global warming, warmer winter conditions allow more species, including hosts and their parasitoids, to be active, resulting in changes to the food-web structure (Tougeron et al. 2018a, 2020). A particular consequence of these warmer winter conditions could be modifications to the overwintering strategies of parasitoids (Hance et al. 2007; Brose et al. 2012). For example, Tougeron et al. (2017) showed that there has been a decrease in the expression of the diapause strategy across the whole guild of parasitoids in the western part of France as a consequence of recent warmer winters, which has, in turn, modified food webs (Tougeron et al. 2018a). In the same way, the aphid–parasitoid community structure can be partly predicted by climatic conditions, promoting thermal niche separation (Woodcock and Vanbergen 2008; Le Lann et al. 2011a; Andrade et al. 2016). Nevertheless, parasitoids and aphids are not alone in the agroecosystem: hyperparasitoids may disrupt the parasitoid-mediated natural control of aphids (Gagic et al. 2011), becoming active on cereal fields during the winter, as a result of a decrease in the number of days below zero (Tougeron et al. 2018a; Tougeron and Tena 2019). Interacting network approaches can help us to understand how natural enemies, such as parasitoids, may control crop host pests efficiently (Tylianakis and Binzer 2014) by linking the food-web complexity to the resilience and stability of ecosystems under exposure to environmental stressors (van Baaren et al. 2020). Such approaches may also help to predict the ecological consequences of climate change (Boukal et al. 2019) during winter, although most studies have focused on the growth/spring season (see Tougeron et al. 2017, 2018b, a, 2020; Damien et al. 2017).

The relation between the food-web structure and species composition depends on the mechanisms governing the overall relationship between climatic severity and the relative abundance of each species (Maunsell et al. 2015; Tougeron et al. 2018a). Therefore, food webs may differ among locations on a given environmental gradient (Tylianakis et al. 2007;

Dyer and Coley 2009; Frank et al. 2018; van Baaren et al. 2020). In this study, we evaluated the variation in species number and interactions within a guild of cereal aphids, their parasitoids, and hyperparasitoids along a latitudinal gradient in Chile during winter. This region is characterized by not possessing a clear north–south gradient in temperature, with areas presenting colder days than others along a transversal gradient (Alfaro-Tapia et al. 2021). We tested the following predictions: (1) The number of species expressing diapause and the rate of diapause within a species is higher in cold than warm areas, in turn influencing interactions within food webs; (2) the diversity and abundance of aphid and parasitoid species that are active during the winter increases in warmer areas; (3) a decrease in parasitoid activity during the winter in the coldest areas (Hance et al. 2007; Péré et al. 2013) decreases the diversity of interactions (Shannon diversity of interactions index), affecting the proportion of possible links for each host–parasitoid pair species (connectance) and the symmetry of the network (web asymmetry). Likewise, declines in the weighted mean number of aphids used by each parasitoid species (generality) and the mean number of parasitoid species attacking each aphid species (vulnerability) should be observed. Finally, since there is greater stability and less variation in the species composition in warm areas (Cirtwill et al. 2015), the level of specialization (H2) should be greater in cold areas.

## Material and methods

### Study site

The study area covered a north–south latitudinal gradient of around 1200 km in the central-south valley of Chile between 29 °S and 40 °S. It included nine localities (Figure 2.1. A) and was conducted over one to three consecutive years, depending on the sampling availability at each of the years and localities: La Serena in 2016 (N=4 fields); Rancagua in 2016 (N=3) and 2018 (N=3); Talca in 2016 (N=12), 2017 (N=6), and 2018 (N=9); Chillan and Pinto in 2018 (N=3); Yungay in 2016 (n=4), 2017 (N=3) and 2018 (N=3); Temuco in 2017 (N=4) and 2018 (N=4); Cunco in 2016 (N=5), 2017 (N=4), and 2018 (N=3); and Osorno in 2018 (N=4). The central-south valley is characterized by arable fields, mostly containing annual crops (2016/17 season). In terms of cereal production, approximately 39% is carried out in the La Araucanía region, 24% in the Biobío region, and 14% in the Maule region. The main cereal crops produced in this area (from Coquimbo region to Los Lagos region in Chile) are grown in a total of 395,556 ha. These include wheat *Triticum aestivum* (56.89%), oat *Avena sativa* (34.59%), barley *Hordeum vulgare* (3.43%), and triticale (*Triticosecale* Wittm. ex A. camus) and rye (*Secale cereale*) (5.09%) (ODEPA 2017). This transversal valley is located between two mountain ranges with a north–south disposition, the Andes along the eastern border, and the Costa range along the shore in the west (Luebert and Pliscoff 2018).

## Meteorological data

Meteorological data were obtained from June to September for the three years of sampling (2016–2018) from the meteorological stations (https://www.agromet.cl/) nearest to each of the nine localities. Each year, the mean daily temperature (mean), minimal (Tmin) and maximum temperatures (Tmax) as well as the total number of hours with temperatures below zero ($h < 0$ °C), which is close to the developmental threshold of most *Aphidius* species (Sigsgaard 2000), were calculated (Table S2.1 in Appendix 2.1). Temperature is a main driver of diapause and fluctuations in the occurrence and abundance of the trophic networks of aphid and parasitoid species (Tougeron et al. 2018a).

## Insect sampling

Two sampled dates between July and August (mid to the end of winter of the southern hemisphere) of each year, aphids and aphid mummies (dead aphid containing a developing parasitoid pupa) were randomly collected from cereal crops (wheat and barley) from an area of 1000 m$^2$ over a 40 min period by two people (following the protocol of Andrade et al. 2016). Fields at each locality were separated one from another by at least 500 m, and field borders were excluded to avoid potential margin effects. Living aphids were taken from the field and maintained in the laboratory at 20 °C under a 16:8 h photo regimen (L:D) on winter wheat for two weeks to wait for the potential mummification of each individual sampled. Mummies were maintained individually in 1.5 mL Eppendorf tubes under laboratory conditions until their emergence. Following their emergence, parasitoids and hyperparasitoids were freeze-killed at –20 °C and identified using morphological features (Starý 1995; Tomanović et al. 2014; Hullé et al. 2020) under an optical stereo microscope. Hyperparasitoids were identified at the genus level. Emergences from aphid mummies were recorded daily. Mummies that did not emerge for 25 to 30 days after the last emergence were then dissected, and their contents were identified as dead immature or adult parasitoids or a diapausing parasitoid prepupae (golden-yellow plump pre-pupa) (see pictures and description in Tougeron et al. 2017). Diapausing individuals were preserved in 95% ethanol for further analyses (see below).

## Parasitoid diapause identification

The presence of diapausing individuals was observed after dissection of the mummies. In order to determine the species' identities, total DNA was extracted using a "cells and tissues" DNA isolation kit (Norgen Biotek Corp., ON, Canada) following the manufacturer's instructions. A fragment of the COI gene was amplified from the parasitoid DNA using the universal invertebrate primers LCO-1490 and HCO-2198 (Folmer et al. 1994). PCR amplification was performed with a final volume of 25 µl containing 15 µl of the master mix, which included 0.25 µl Taq DNA Polymerase (5U/µl), 2.5 µl of buffer 1X, 0.2 mM dNTP, 3 mm $MgCl_2$, 8.25 µl of $ddH_2O$, 10 µl DNA, and 1 µM of each primer plus. The PCR cycling profile comprised an initial heating period of 95 °C for 4 min, followed by 36 cycles of 94 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min; 1 cycle of 72 ° C for 5 min; and a final extension step of 15 °C to infinity. PCR products were stored at 4 °C until their visualization in 1.5% agarose gel. All PCR products were sent to Macrogen Inc (Seoul, Korea) for purification and bidirectional sequencing. Once obtained, the DNA sequences were edited using BioEdit sequence alignment editor v7.2.5 (Hall 1999) to generate a consensus sequence for each specimen. Finally, the sequences were compared and analyzed by BLASTn on NCBI (https://www.ncbi.nlm.nih.gov/) and the Barcode of life data system v4 (https://www.boldsystems.org/). The species were determined by similarity alignment (≥98%) of the sequences of the diapausing individuals in the library.

## Quantitative food-web metrics

Two trophic levels were considered in the food-webs: the first included aphid species and the second included primary parasitoids and hyperparasitoids, which were placed at the same level as it was not possible to determine the primary parasitoid that the hyperparasitoids emerged from. Quantitative food webs were constructed for each field and for nine localities based on pooled data. Quantitative network metrics were measured in accordance with Tylianakis et al. (2007) using the *bipartite* R package (Beckett et al. 2020). We calculated the following metrics in accordance with Maunsell et al. (2015) and Tougeron et al. (2018): (1) *Shannon's diversity of interactions*, which represents the total number of interactions among species within dynamic aphid–parasitoid communities. For each species, the diversity was measured as the diversity of host inflow from each parasitoid species and the diversity of outflow from each host to its parasitoids; (2) *Connectance*, which represents the proportion of possible links actually observed in a food web and is calculated as the quantitative linkage density divided by the number of species in the network, e.g., hosts vs. parasitoids; (3) *Web Asymmetry*, which represents the balance between the numbers of parasitoid and aphid species. Negative values indicate that there are more species in higher than in lower trophic levels; (4) H2, which indicates the level of specialization within a network (0 = no specialization to 1 = perfect specialization); (5) *Generality*, which is the weighted mean number of host species (aphids) per consumer species (parasitoids); and (6) *Vulnerability*, the weighted mean number of consumers (parasitoids) per host species (aphids).

## Statistical analysis

First, a non-metric multidimensional scaling (NMDS) analysis was employed to assess group localities and years by climatic similarities based on a distance matrix using the metaMDS function in the "vegan" package (Oksanen et al. 2020), and the stress values were used as measurements of goodness of fit of data. Values equal to or less than 0.20 were considered meaningful for the analysis, in accordance with Clarke (1993). After that, a Principal Component Analysis (PCA) was performed to represent these categories, as done by Tougeron, Damien, et al. (2018). Then, using the *bipartite* package (Beckett et al. 2020), we built quantitative food webs showing the parasitoid and aphid community compositions in different localities/years during the winters of 2016 to 2018 along the environmental gradient, and the Food Web Designer v3.0 software (Sint and Traugott 2016) was used to plot the trophic interaction networks. Subsequently, a Generalized Linear Model (GLM) assuming a negative binomial distribution with a log-link function was used to evaluate the effects of environmental factors on the community composition by comparing the patterns of aphid and parasitoid abundance in relation to climate variables. When evaluating the effect of temperature on the abundance of aphid and parasitoid species in each locality and year of sampling, the mean of Tmin and mean number of hours below 0 °C were used as explanatory variables. We chose these variables because they are close to the freezing temperature and below the developmental threshold of most parasitoid species (Sigsgaard 2000). Additionally, they have great influences on the aphid abundance during the winter (Honek et al. 2018) and may be good predictors of the diapause frequency (Tougeron et al. 2018b). In addition to climatic variables, the effect of host resources on the presence of parasitoid species was evaluated, where the abundance of aphid species was used as an explanatory variable. Only the main primary parasitoids were considered for this analysis, and the *A uzbekistanicus* and *A. rhopalosiphi* complex (*A. uzb/rho* from here on) was considered a complex of species, as these species are closely related and are considered sister species (Kos et al. 2011). Uncommon parasitoid species, representing less than 5% of the samples over the three-year period, were excluded from this analysis.

Finally, the effect of the winter conditions determined on the quantitative food web metrics (Shannon's diversity of interactions, Connectance, Web Asymmetry, H2, Generality and Vulnerability) were tested using GLMs assuming a Gaussian distribution and an identity-link function. Since quantitative food web metrics are strongly affected by the network size (Morris et al. 2004), the log of the total abundance of recorded insects was included as a covariate (Maunsell et al. 2015). For all analyses, the best model was chosen by using the Akaike information criterion and performing an ANOVA type II using the *car* package (Fox et al. 2016). Pairwise comparisons were done using 'Tukey tests', correcting for multiple comparisons with the 'single-step' method using the *Multcomp* package (Hothorn et al. 2016).

All the statistical analyses were conducted with the R 3.6.5 statistical software (R Core Team 2019).

# Results

## Environmental gradient determination

Our analysis showed a final stress value of 0.02 and a linear fit with $R^2 = 0.99$. The NMDS ordination results showed inter locality/year variability depending on the meteorological variables. Talca 2018, Pinto 2018 and Cunco 2016/17/18 were grouped as ‘cold winter localities’; Rancagua 2016/18, Talca 2016/17, Chillan 2018, Temuco 2017/18, and Osorno 2018 were grouped as ‘mild winter localities’; and La Serena 2016/17/18 was a ‘warm winter locality’. In addition, the PCA representation showed that the coldest winter localities were characterized by having more hours below zero (h<0°C), and the warmest winter localities had the highest mean minimum temperatures (Figure 2.1. B).

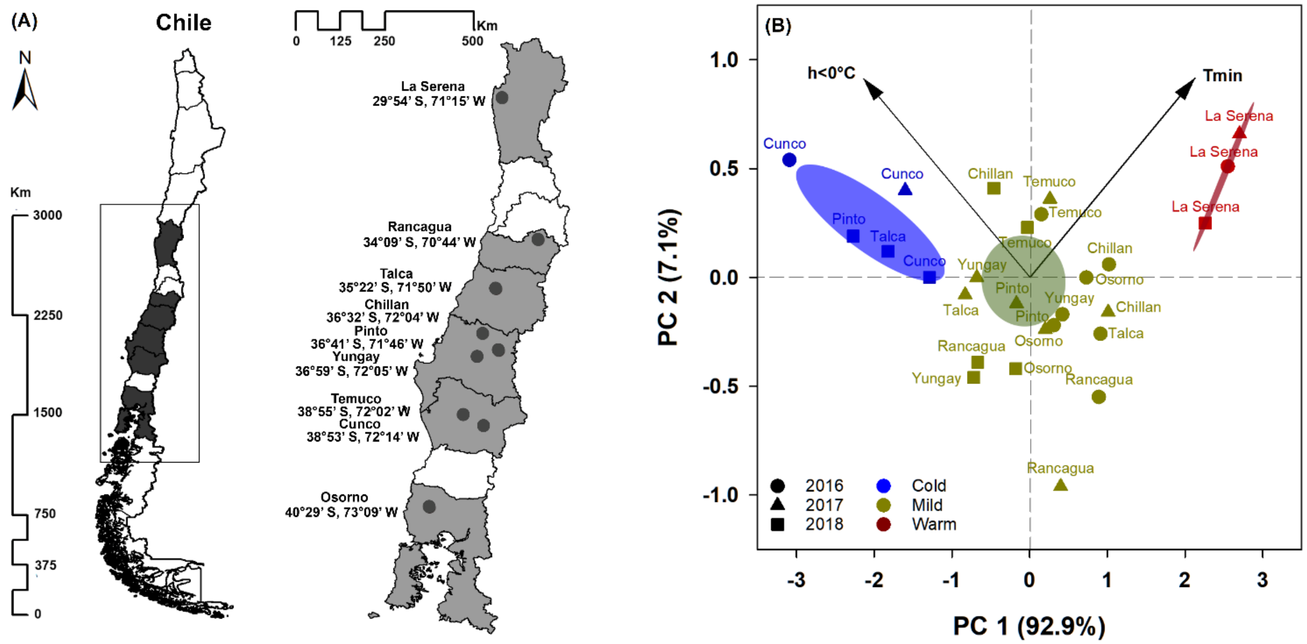

**Figure 2.1.** (A) Map showing the nine localities where aphids and parasitoids were sampled during three consecutive years (2016–2018) along a latitudinal gradient in the central-south valley of Chile and (B) Principal components analysis (PCA) biplot based on climatic variables (mean minimal temperature 'Tmin' and number of hours below zero

## Aphid and parasitoid food web composition and rate of diapausing individuals

Across the winters of 2016 to 2018, we recorded a total of 6899 aphids, including living aphids and mummies, from four species identified as *Rhopalosiphum padi* (70.26%), *Sitobion avenae* (21.67%), *Metopolophium dirhodum* (7.23%), and *Schizaphis graminum* (0.84%) (Hemiptera, Aphididae). Out of the 1493 recorded mummies, 68.72% were emerged individuals, 27.46% were non-emerged (dead larva/adult individuals), and 4.22% were diapausing individuals, including three parasitoid (2.52%) and one hyperparasitoid species (1.70%). Across all localities and years, parasitoid assemblages were dominated by primary parasitoids such as *Aphidius platensis* (61.60% of the total number of emerged individuals), *Aphidius ervi* (12.96%), and the *A. uzb/rho* complex (11.50%). *Aphidius avenae*, *Aphidius* *matricariae*, *Praon volucre*, *Lysiphlebus testaceipes,* and *Ephedrus* sp. (Hymenoptera, Braconidae) constituted only 6.82% of the total number of emerged individuals. We also recorded 73 hyperparasitoid individuals identified as *Phaenoglyphis* sp, *Alloxysta* sp (Hymenoptera, Figitidae), *Dendrocerus* sp (Hymenoptera, Megaspilidae), and *Asaphes* sp (Hymenoptera, Pteromalidae) and one unidentified species (all hyperparasitoids together represented only 7.12% of all emerged individuals). The total numbers of aphids, aphid mummies, emerging parasitoids, and hyperparasitoids are summarized in Table 2.1. The relative abundance of species within the guilds of aphids and parasitoids and the food web structure differed across the locality/year gradient (see Figure. 2.2). There was a high level of variability in the relative proportions of aphids between localities/years. The proportion of *R. padi* among all aphids was higher in warm winter areas than, in mild, and cold winter areas respectively. The second-most-abundant aphid species was *S. avenae,* with low relative abundances in warm winter areas, follow of mild, and cold winter areas respectively. Additionally, *M. dirhodum* was not found in warm areas, while in mild and cold winter areas, it represented similar relative abundance. *S. graminum* represented less than 4.0% of parasitized aphids in all areas determined. Within the parasitoid guild, the species with the highest levels of abundance also had the broadest geographic distributions, whereas the less frequent ones were limited to a single year or locality, and important inter-annual variations were observed. The generalist parasitoid *A. platensis* was observed across the environmental gradient (except in Osorno, the southernmost locality) with a higher relative abundance of in warm, mild, and cold winter areas respectively. This parasitoid species was mainly associated with the aphid *R. padi*. *Aphidius ervi*, and the complex *A. uzb/rho* was mostly associated with two aphid species, *S. avenae* and *M. dirhodum*. The relative abundance of *A. ervi* was lower in warm than in mild and cold winter areas, and the relative abundance of *A. uzb/rho* was lower in mild than cold winter areas respectively. Finally, together, *A. avenae*, *A. matricariae*, *Praon* spp., *L. testaceipes*, and *Ephedrus* sp. were observed to have a relative abundance of around 8.0% in mild winter areas, and the relative abundance of *A. matricariae* and *Praon* spp. together in cold winter areas was 3.0%. The four hyperparasitoid genera were also detected to have higher relative abundance in mild winter areas than in cold winter areas but were not recorded in warm winter areas (Figure 2.2).

## Diapausing parasitoid and hyperparasitoid species in mild and cold localities

The proportion of diapausing individuals was 3.5%, 4.3%, and 3.9% in 2016, 2017, and 2018, respectively (Table 2.1). In general, all diapausing species were recorded in both mild and cold winter localities (Table 2.2). Localities/years where no diapausing individuals were recorded were La Serena in 2016 and Chillan, Yungay, and Cunco in 2018. Three parasitoid species, *A. ervi, A. uzbekistanicus*, and *P. volucre*, and one hyperparasitoid species, *Phaenoglyphis villosa*, were found in diapause (Table 2.2). In 2016, all individuals in diapause were from the hyperparasitoid species *P. villosa* and were found on *R. padi*, *S. avenae*, and *S. graminum*. Of all the hyperparasitoids identified, three came from *Praon* sp. (two on *S. avenae* and one on *R. padi*) and were identified by the characteristic mummy of this genera. In the samples from the years 2017 and 2018, only primary parasitoid species were found in diapause. In 2017, we recorded ten *A. ervi* individuals on *R. padi* (1), *S. avenae* (6), and *M. dirhodum* (3). Two *A. uzbekistanicus* individuals were identified on *R. padi*, and five *P. volucre* individuals were found on *R. padi*. Finally, in 2018, we recorded five *A. ervi* individuals on *S. avenae* (4) and *M. dirhodum* (1), five *A. uzbekistanicus* individuals on *R. padi* (2), *S. avenae* (1), and *M. dirhodum* (2), and seven *P. volucre* individuals on *R. padi* (6) and *S. avenae* (1) (Table 2.2).

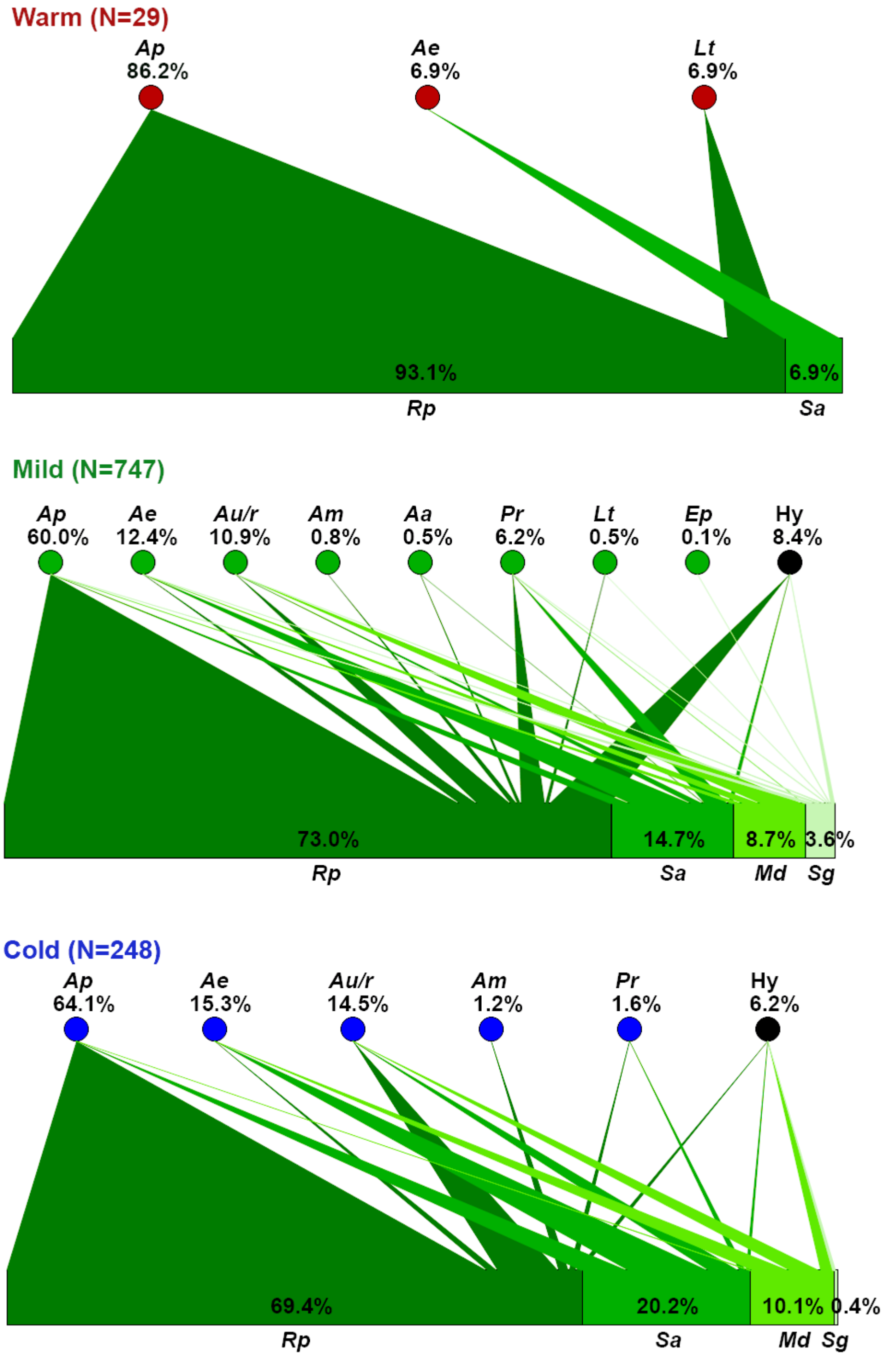

**Figure 2.2.** Quantitative aphid–parasitoid food webs in the central-south valley of Chile. Horizontal bars represent the relative abundance of each aphid species (lower) and their parasitoids, including hyperparasitoids (upper points). Arrows represent the interaction strength (% of relative abundances) between host–parasitoid pairs.

**Active aphids:**

*Rhopalosiphum padi* (*Rp*); *Sitobion avenae* (*Sa*); *Metopolophium dirhodum* (*Md*); *Schizaphis graminum* (*Sg*).

**Active parasitoids:** *Aphidius platensis* (*Ap*); *Aphidius ervi* (*Ae*); *Aphidius uzb/rho* (*Au/r*); *Aphidius matricariae* (*Am*) *Aphidius avenae* (*Aa*); *Praon* spp. (*Pr*); *Lysiphlebus testaceipes* (*Lt*); *Ephedrus* sp. (*Ep*).

**Active hyperparasitoids (Hy):** *Phaenoglyphis* sp + *Alloxysta* sp + *Dendrocerus* sp + *Asaphes*

**Table 2.1.** Total number of fields sampled and frequency of cereal aphids, mummies, emerged parasitoids, hyperparasitoids, non-emerged parasitoids (dead larva/adult individuals), and diapausing individuals during winter on cereal crops in the central-south valley of Chile for each locality and year. Localities are presented from North (top of the table) to South (bottom of the table). The total percentage of diapausing individuals in each location and year is shown in parentheses.

| | **2016** | | | | | | | **2017** | | | | | | | **2018** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Locality** | **Number of fields** | **Live aphids** | **Mummies** | **Emerged parasitoid** | **Hyperparasitoids** | **Non-emerged** | **Diapausing** | **Number of fields** | **Live aphids** | **Mummies** | **Emerged parasitoid** | **Hyperparasitoids** | **Non-emerged** | **Diapausing** | **Number of fields** | **Live aphids** | **Mummies** | **Emerged parasitoid** | **Hyperparasitoids** | **Non-emerged** | **Diapausing** |
| **La Serena** | 4 | 214 | 53 | 29 | 0 | 24 | 0 (0.0) | | | | | | | | | | | | | | |
| **Rancagua** | 3 | 241 | 126 | 66 | 0 | 60 | 4 (3.2) | | | | | | | | 3 | 64 | 22 | 15 | 0 | 7 | 1 (4.5) |
| **Talca** | 12 | 1054 | 360 | 199 | 39 | 122 | 16 (4.4) | 6 | 607 | 206 | 134 | 6 | 66 | 12 (5.8) | 9 | 678 | 167 | 135 | 2 | 30 | 9 (5.4) |
| **Chillan** | | | | | | | | | | | | | | | 3 | 546 | 114 | 87 | 3 | 24 | 0 (0.0) |
| **Pinto** | | | | | | | | | | | | | | | 3 | 154 | 45 | 38 | 0 | 7 | 3 (6.7) |
| **Yungay** | 4 | 222 | 65 | 30 | 3 | 39 | 1 (1.5) | 3 | 152 | 54 | 34 | 7 | 13 | 1 (1.9) | 3 | 55 | 1 | 1 | 0 | 0 | 0 (0.0) |
| **Temuco** | | | | | | | | 4 | 264 | 83 | 56 | 7 | 20 | 1 (1.2) | 4 | 452 | 32 | 29 | 0 | 3 | 2 (6.3) |
| **Cunco** | 5 | 314 | 61 | 27 | 0 | 34 | 2 (3.3) | 4 | 200 | 52 | 40 | 6 | 6 | 3 (5.8) | 3 | 12 | 0 | | | | |
| **Osorno** | | | | | | | | | | | | | | | 4 | 177 | 52 | 33 | 0 | 19 | 2 (3.8) |
| **Total** | **28** | **2045** | **665** | **351** | **42** | **279** | **23 (3.5)** | **17** | **1223** | **395** | **264** | **26** | **105** | **17 (4.3)** | **32** | **2138** | **433** | **337** | **5** | **95** | **17 (3.9)** |

**Table 2.2.** Parasitoid (*Aphidius ervi*, *Aphidius uzbekistanicus* and *Praon volucre*) and hyperparasitoid (*Phaenoglyphis villosa*) species recorded in diapause during winter on different aphid species in cereal crops in the central-south valley of Chile for each locality and year sampled. The numbers of emerged non-diapausing and diapausing individuals and their respective percentages are shown in parentheses. The remaining percentage corresponds to the non-emerged (dead) individuals.

| | | | | ***A. ervi*** | | ***A. uzbekistanicus*** | | ***P. volucre*** | | ***P. villosa*** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Aphids** | **Mummies** | **Emerged** | **Diapausing** | **Emerged** | **Diapausing** | **Emerged** | **Diapausing** | **Emerged** | **Diapausing** |
| **2016** | **Rancagua** | *R. padi* | 63 | 1 (1.5) | | 1 (1.6) | | 1 (1.6) | | 0 | 1 (1.6) |
| | | *S. avenae* | 14 | 7 (50.0) | | 1 (7.1) | | | | 0 | 1 (7.1) |
| | | *S. graminum* | 19 | 1 (5.2) | | 2 (10.5) | | 1 (5.3) | | 0 | 1 (5.3) |
| | **Talca** | *R. padi* | 309 | 2 (0.6) | | 3 (1.0) | | 10 (3.2) | | 15 (4.9) | 14 (4.5) |
| | | *S. avenae* | 39 | 6 (15.3) | | | | 11 (28.2) | | 0 | 2 (5.1) |
| | **Yungay** | *R. padi* | 53 | 3 (5.7) | | | | | | 0 | 1 (1.9) |
| | **Cunco** | *R. padi* | 31 | 0 (0.0) | | 2 (6.5) | | | | 0 | 2 (6.5) |
| | | *S. avenae* | 23 | 6 (26.0) | | 2 (8.7) | | | | 0 | 1 (4.4) |
| **2017** | **Talca** | *R. padi* | 175 | 2 (1.1) | 1 (0.6) | 3 (1.7) | 2 1.1 | 5 (2.9) | 5 (2.9) | | |
| | | *S. avenae* | 18 | 8 (44.4) | 3 (16.7) | 3 (16.7) | | 4 (22.2) | | | |
| | | *M. dirhodum* | 3 | 0 (0.0) | 1 (33.3) | | | | | | |
| | **Yungay** | *S. avenae* | 8 | 2 (25.0) | 1 (12.5) | | | | | | |
| | **Temuco** | *S. avenae* | 11 | 2 (18.1) | 1 (9.1) | 0 | | 2 (18.2) | | | |
| | **Cunco** | *S. avenae* | 4 | 2 (50.0) | 1 (25.0) | 1 (25.0) | | | | | |
| | | *M. dirhodum* | 7 | 1 (14.2) | 2 (28.6) | 2 (28.6) | | | | | |
| **2018** | **Rancagua** | *R. padi* | 13 | 2 (15.3) | | 0 | | 0 | 1 (7.7) | | |
| | **Talca** | *R. padi* | 138 | 3 (2.2) | | 7 (5.1) | 2 1.5 | 2 (1.5) | 4 (2.9) | | |
| | | *S. avenae* | 26 | 7 (26.9) | 2 (7.7) | 1 (3.9) | 1 3.9 | 2 (7.7) | | | |
| | **Pinto** | *S. avenae* | 15 | 6 (40.0) | 2 (13.3) | 3 (20.0) | | 0 | | | |
| | | *M. dirhodum* | 16 | 6 (37.5) | 1 (6.2) | 5 (31.3) | | 0 | | | |
| | **Temuco** | *R. padi* | 24 | 0 (0.0) | | 4 (16.7) | | 0 | 1 (4.2) | | |
| | | *S. avenae* | 8 | 5 (62.5) | | 0 | | 1 (12.5) | 1 (12.5) | | |
| | **Osorno** | *M. dirhodum* | 45 | 1 (2.2) | | 27 (60.0) | 2 4.4 | 0 | | | |

## Effects of the temperature and host on the community composition

The selected climatic variables (mean of Tmin and hours below 0 °C) were not observed to have significant influences on the abundance levels of *R. padi*, *S. avenae*, or *M. dirhodum*. In the same way, the most abundant parasitoids, *A. platensis*, *A. ervi* and the complex *A. uzb/rho*, were not affected by changes in Tmin or by the mean of h<0 °C (Table 2.3 and Figure S2.2 in Appendix 2.2). In the analysis where aphid hosts were included as fixed factors to explain the parasitoid abundance during winter, the abundance of *A. platensis* (Figure 2.3 A) increased with the abundance of *R. padi*, whereas this species was not affected by the abundance of S. avenae or *M. dirhodum* (Table 2.3). In addition, the abundance of *A. ervi* (Figure. 2.3 B) was not affected by the abundance of *R. padi*; however, it significantly increased as the abundance levels of S. avenae and *M. dirhodum* increased (Table 2.3). Finally, the abundance of the complex *A. uzb/rho* (Figure 2.3 C) was positively linked to the abundance levels of *R. padi*, *S. avenae*, and *M. dirhodum*.

## Quantitative food web metrics across the determined winter conditions

There were no significant differences in any of the food web metrics tested (Figure 2.4). The Shannon's diversity of interactions (GLM: $\chi^2 = 3.90$; *df* = 2, *P* = 0.14), connectance (GLM: $\chi^2 = 1.96$; *df* = 2, *P* = 0.37), web asymmetry (GLM: $\chi^2 = 0.18$; *df* = 2, *P* = 0.91), vulnerability (GLM: $\chi^2 = 2.76$; *df* = 2, *P* = 0.25), generality (GLM: $\chi^2 = 0.06$; *df* = 2, *P* = 0.97), and H2 index (GLM: $\chi^2 = 0.86$; *df* = 2, *P* = 0.65) were not affected by the winter conditions.

**Table 2.3.** Results of model selection for the main aphid and parasitoid species present in winter. The influence of temperature (Tmin and h<0°C) on the abundance of the dominant aphid species, *Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*, and the dominant parasitoid species, *Aphidius platensis*, *Aphidius ervi* and the complex *Aphidius uzbekistanicus/rhopalosiphi*, was tested. The influence of the exploited aphid species on the abundance of each parasitoid species was tested. Columns correspond to the tested dependent variables, the AIC value, and the estimate values determined in the analysis. Statistically significant p-values (≤0.05) are presented in bold.

| **Selected model: Aphids sp ~ Tmin + h<0°C** | | | | | |
|---|---|---|---|---|---|
| **Dependent variable** | **Parameter** | **AIC** | $\chi^2$ | ***Df*** | ***p-value*** |
| *R. padi* | Tmin | 777.62 | 3.42 | 1 | 0.06 |
| | h<0°C | | 0.86 | 1 | 0.35 |
| *S. avenae* | Tmin | 596.83 | 0.26 | 1 | 0.61 |
| | h<0°C | | 1.82 | 1 | 0.18 |
| *M. dirhodum* | Tmin | 374.54 | 2.88 | 1 | 0.09 |
| | h<0°C | | 1.08 | 1 | 0.29 |
| **Selected model: Parasitoids sp ~ Tmin + h<0°C** | | | | | |
| *A. platensis* | Tmin | 477.53 | 0.04 | 1 | 0.85 |
| | h<0°C | | 0.07 | 1 | 0.80 |
| *A. ervi* | Tmin | 271.59 | 0.01 | 1 | 0.92 |
| | h<0°C | | 0.18 | 1 | 0.67 |
| *A. uzb/rho* | Tmin | 238.19 | 0.22 | 1 | 0.64 |
| | h<0°C | | 0.12 | 1 | 0.72 |
| **Selected model: Parasitoids sp ~ R. padi + S. avenae + M. dirhodum** | | | | | |
| *A. platensis* | *R. padi* | 451.60 | 30.26 | 1 | **< 0.001** |
| | *A. avenae* | | 1.33 | 1 | 0.25 |
| | *M. dirhodum* | | 0.27 | 1 | 0.60 |
| *A. ervi* | *R. padi* | 239.30 | 3.68 | 1 | 0.05 |
| | *A. avenae* | | 7.37 | 1 | **0.01** |
| | *M. dirhodum* | | 14.57 | 1 | **< 0.01** |
| *A. uzb/rho* | *R. padi* | 197.53 | 5.45 | 1 | **0.02** |
| | *A. avenae* | | 5.56 | 1 | **0.02** |
| | *M. dirhodum* | | 53.16 | 1 | **< 0.001** |

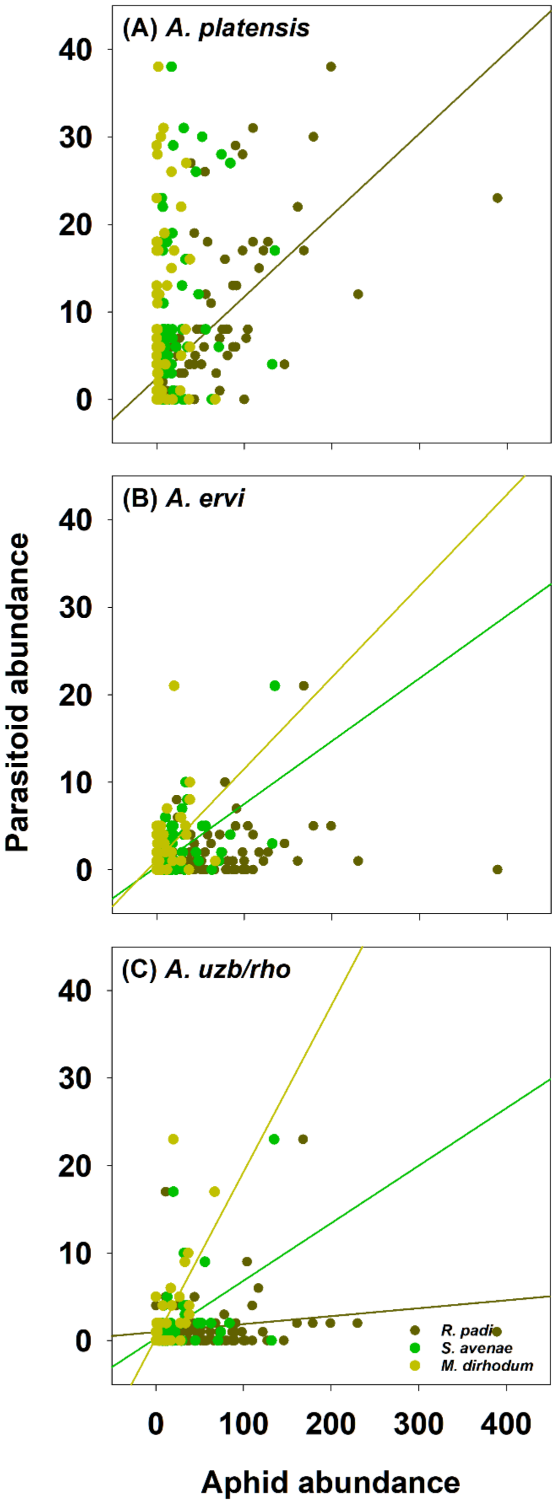

**Figure 2.3**. Generalized linear model results showing the relationship between the abundance per field for the main active aphid species (*Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*) and the abundance of the main active parasitoid aphid species: A) *Aphidius platensis* B) *Aphidius ervi* and C) *Aphidius uzb/rho.* The trend line shows the significant links between aphid species and parasitoids based on the negative binomial regression.

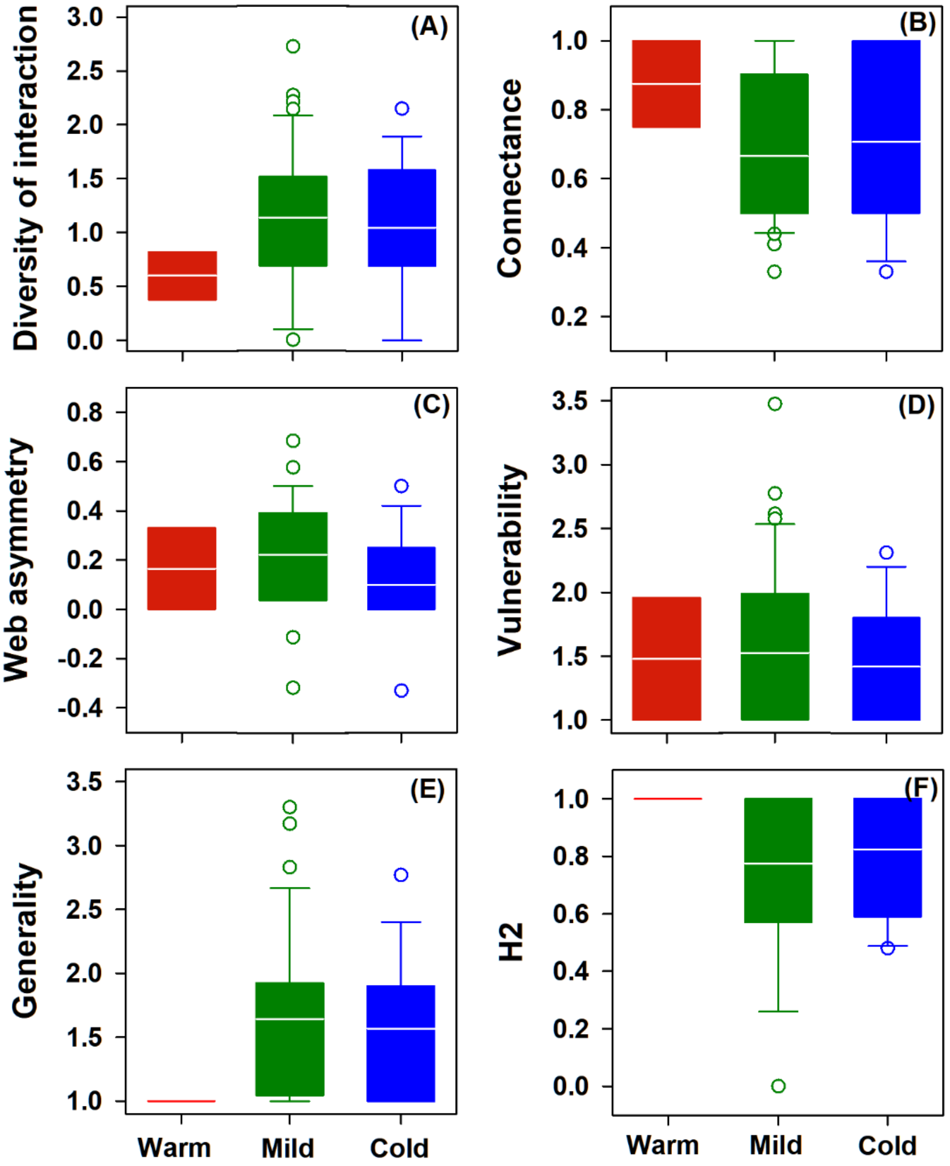

**Figure 2.4.** Box plot of the gradient (warm, mild and cold winters areas) showing (A) Shannon´s diversity of interaction; (B) Connectance, the overall complexity of the food web (realized proportion of potential links); (C) Web-asymmetry, the balance between the numbers of parasitoid and aphid species (negative values indicate more species in higher than in lower trophic-level); (D) Vulnerability, the weighted mean number of parasitoid species attacking a given aphid species; (E) Generality, the weighted mean number of aphid species exploited by each parasitoid species; and (F) ´H2´, the level of specialization within a network, from 0 (no specialization) to 1 (perfect specialization). There were no differences among climatic areas for the evaluated metrics.

## Discussion

In this study, we described aphid–parasitoid food webs based on an extensive three-year dataset collected from nine localities. Despite the absence of a clear north–south latitudinal temperature gradient in the central-south Chilean valley, we managed to classify localities/years as either cold, mild, or warm winter areas. According to our results, the initial hypothesis that there was a cline in diapause expression was rejected. Only low levels of diapause incidence (around 4%) were observed in each of the three years of evaluation across the whole geographic gradient. Diapause only occurred in three of the nine parasitoid species, *A. ervi*, *A uzb/rho* and *P. volucre*, and one hyperparasitoid species, *P. villosa*. Given that these species are not among the four most abundant species, and as the rate of diapause of the most abundant species *A. platensis* was null (see also Alfaro-Tapia et al. 2021), the total level of diapause did not vary among climatic areas. Nevertheless, we observed changes in the compositions of aphid and parasitoid species, as their levels of relative abundance varied among the climatic areas, with fewer aphid and parasitoid species in warm winter areas than in mild and cold winter areas. The food webs were mainly dominated by *R. padi* and its parasitoid *A. platensis,* but we did not find support for our hypothesis concerning the six food web metrics analyzed. This suggests that environmental filtering in the warm, mild, and cold winter areas in central-south Chile does not influence the aphid–parasitoid trophic networks. This could be explained by the fact that the winters in the central-south valley of Chile are not cold enough than in other latitudes. In addition, this region suffers from relative interannual homogenization in temperature as a consequence of climate change. Specifically, there has been an increase in temperature in the Andes (+0.25°C/decade) (Falvey and Garreaud 2009) and an increase in the mean annual temperature of between 10-15°C in low and mid elevation zones (0–1000 m.) (Zambrano-Bigiarini et al. 2017).

Our results show that most adult individuals within the parasitoid guild are active during winter. The low level and/or the absence of diapause (e.g., for *A. platensis*) may be due to the overall mild winter temperatures in the central-south valley of Chile, which do not reach the necessary threshold to trigger diapause in aphid parasitoids, as observed by Tougeron et al. (2017, 2018b) in cereal aphid–parasitoids from the west part of France. In our previous study

(Alfaro-Tapia et al. 2021), *A. platensis* did not express diapause under fall and winter-like laboratory conditions. However, under the same conditions, we observed low levels of diapause in *A. ervi* (around 5%) on cereal aphids (Chapter IV), although these conditions usually induce a high level of diapause in other *Aphidius* parasitoid species in areas with cold winter conditions (Brodeur and McNeil 1989; Tougeron et al. 2018b). This suggests that either the conditions have never been cold enough in Chile since the introduction of this species to require diapause or that recent winter warming has led to a decrease in diapause. Parasitoids were introduced in the 1970s from France (e.g., *A. ervi*, *A uzbekistanicus*, *A. rhopalosiphi, P. volucre*) (Zepeda-Paulo et al. 2016) and the United States (e.g., *L. testaceipes)* as part of classical biological control programs; however, other species may have been accidentally introduced on unknown dates and from unknown origins (e.g., *A. platensis*) (Starý et al. 1993). The low levels of diapause found in the studied populations may also be a response to continuous host availability throughout the winter season, since *R. padi*, *S. avenae* and *M. dirhodum* were all found to be present during the winter. In addition, we found a few individuals of only one hyperparasitoid species in diapause in one year, whereas the other hyperparasitoid species (i.e., *Alloxysta* sp, *Dendrocerus* sp, *Asaphes* sp) were recorded as being active every year, providing the first evidence that a large portion of the hyperparasitoid community is active during the winter in Chile.

In our study, the minimum temperature and the total number of hours below 0 °C over the winter did not explain the aphid species composition. The distribution of aphid species was not changed by the environmental temperature in the central-south valley of Chile. In areas characterized by mild winter conditions, the absence of lethal frosts allows aphids to remain active, leading to a dominance of parthenogenetic genotypes (Langer et al. 1997; Dedryver et al. 2001; Andrade et al. 2015). However, minimal temperatures and the mean duration of frost events are good predictors of aphid abundance (Honek et al. 2018; Tougeron et al. 2018a), as low temperatures slow down the development and reduce the survival of aphids (Brabec et al. 2014). The difference between this finding and our results could be explained by the lack of frequent frost events during the study period in the central-south valley of Chile. Moreover, *R. padi* is the most abundant aphid species in winter across the environmental gradient, and it has been reported to harbor an obligate parthenogenetic reproductive mode in Chile (Rubio-

Meléndez et al. 2019), allowing its persistence in winter temperatures and its adaptation to a large range of local conditions (Figueroa et al. 2018; Simon and Peccoud 2018). Similar patterns in the aphid species composition have been observed during winter in the west of France, an area that is also dominated by *R. padi* (Alford et al. 2014; Andrade et al. 2016). *R. padi* usually shows greater cold tolerance than other cereal aphid species (Alford et al. 2014, 2018), providing it with a fitness advantage during winter. At the same time, it is also considered to be the strongest competitor during the plant vegetative growth stage of cereal crops, displacing other species of aphids (Gianoli 2000). Conversely, *S. avenae* and *M. dirhodum* were also recorded to have lower levels of abundance during winter, increasing their dominance towards the beginning of spring. The variation of cool and humid to warm and dry conditions could explain this pattern (Alford et al. 2014).

Usually, winter is stressful for parasitoids, as temperatures come close to developmental and activity thresholds (Sigsgaard 2000; Zamani et al. 2007). It has been demonstrated that the temperature is fundamental for determining the temporal and geographical distribution of a species (Thomas et al. 2004; Boher et al. 2012), through its diapause expression (Tougeron et al. 2018a), thermal tolerance (Le Lann et al. 2011b), and the thermal tolerance of closely interacting species, such as aphid hosts (Alford et al. 2014). In our study, the minimum temperature and number of hours below 0°C were also not found to be good predictors of parasitoid abundance, probably because the winters are not cold enough. The parasitoid guild was smaller in warm winter areas (only *A. platensis, A. ervi*, and *L. testaceipes* were recorded) than in mild and cold winter areas with inter locality/year variability in terms of the species number and relative abundance. This is likely because the proportion of cereal fields in the landscape in the northern zone (areas with warmer winters) is lower than in other areas (ODEPA 2017). Moreover, climatic differences in the surveyed area may be an important factor that influences the local and seasonal availability of the host plants and aphids. Thus, the species composition and relative abundance of parasitoids will also be affected (Starý et al. 1993). Overall, the dominant parasitoid species (*A. platensis*) was found to exploit all recorded aphid species with significant levels of relative abundance. These results are supported by the high cold acclimation capacity observed in populations of *A. platensis* in Chile and their reasonably high fecundity at low temperatures (Chapter III, Alfaro-

Tapia et al. 2021). On the other hand, *A. platensis*, together with *A. ervi* and *A. uzb/rho*, was found to constitute a large proportion of parasitoids in mild and cold winter areas that were mainly associated with *S. avenae* and *M. dirhodum*. Consequently, the changes in temperature over the study area did not explain the relative abundance of parasitoids. In contrast, our results showed compartmentalization of winter food webs through bottom-up effects, as the parasitoid abundance was directly explained by the aphid abundance (Andrade et al. 2016; Tougeron et al. 2018a). Indeed, the environmental distribution of parasitoids depends on the host plants and associated aphids present (Le Ralec et al. 2010). In addition, hyperparasitoids were observed to be active during winter and were most abundant in 2016 and 2017 (around 6% of the total emerged individuals). As previously reported elsewhere, hyperparasitoids are common in many host–parasitoid networks (Tena et al. 2008; Lohaus et al. 2013). These species can only be active in winter if their host, the primary parasitoids, is also available which, in turn, requires the presence of the aphid hosts (Tougeron and Tena 2019). Therefore, mild winter conditions could allow the development and activity of hyperparasitoids in Chile. This scenario has been also observed in the west of France, where the community of aphids and parasitoids has included active hyperparasitoids in winter since 2013 (Andrade et al. 2016; Tougeron et al. 2017). This has also been observed in other aphid–parasitoid communities in Citrus, where hyperparasitoids put a high level of parasitism pressure on the primary parasitoids year-round (Gómez-Marco et al. 2015).

Variation in the relative abundance of parasitoid species and changes in the environmental conditions modify the quality and quantity of resources (Stilmant et al. 2008; Honek et al. 2018). Overall, our analyses of quantitative food web metrics did not reveal marked changes in the interaction structure of cereal aphids and their associated parasitoids in relation to the winter condition along the central-south valley of Chile. The abundance of species usually accurately predicts network metrics at all levels (Canard et al. 2014), but our analysis offers no evidence for consistent variation in Shannon's diversity of interactions based on winter conditions, probably because most of the trophic interactions only occur between a few aphid species and a small number of parasitoid species (Maunsell et al. 2015). Another explanation could be related to the low diversity of species and interactions normally occurring in more intensively managed agricultural fields compared with natural sites

(Tylianakis et al. 2007). We did not find a strong level of support for the presence of a winter effect from the connectance of the studied networks. In our study, one generalist parasitoid, *A. platensis*, dominated the complete study system. This species is well adapted to the winter conditions of Chile (Alfaro-Tapia et al. 2021). No differences were found along the determined winter conditions, probably because the high number of *A. platensis–R. padi* interactions affects the interaction level. A similar pattern was observed in a rainforest stratum where the generalist species *Melittobia australia* (Hymenoptera, Eulophidae) dominates the system parasitizing *Fabriogenia* sp. (Hymenoptera, Pompilidae), rendering the connectance metric constant among different strata (Morris et al. 2015). Likewise, the balance between the number of parasitoids and aphid species ´Web asymmetry´ did not differ along the gradient, showing values close to zero. Therefore, the numbers of aphids and parasitoids were symmetric along the gradient, involving approximately the same numbers of aphid and parasitoid species. This result is similar to what was found in a long-term winter study carried out in the west of France, where the numbers of aphids and parasitoids in quantitative food webs were similar over years (Andrade et al. 2016; Tougeron et al. 2018a). Both aphids and their parasitoids are mostly active in cereal crops of Chile in the winter, as has also been demonstrated in countries with mild winter climates (Polgár et al. 1995; Andrade et al. 2016; Tougeron et al. 2017, 2018a).

In accordance with our hypothesis, generality did not increase under warm winter conditions. The main parasitoid species recorded, *A. platensis*, *A. ervi*, and *A. uzb/rho*, were found to attack multiple aphid species. This happened despite the availability and relative abundance of their favorite hosts, which accept all aphid hosts encountered, even if they are not optimal (Eoche-Bosy et al. 2016; Alfaro-Tapia et al. 2021). Diverse parasitoid communities have been shown to enhance pest suppression through the complementary use of aphid host resources by niche partitioning (Finke and Snyder 2008; Andrade et al. 2016) via the addition of new species to the food web (Tougeron et al. 2018a). This niche overlapping was also observed in spring in the same system in the Central valley of Chile among *A. ervi*, *A. uzbekistanicus*, and *A. rhopalosiphi* (Ortiz-Martínez et al. 2019). In the same way, the climatic conditions were found to have no influence on the mean number of aphid parasitoids per host species (vulnerability). This suggests that parasitoids adjust their attack rate on each aphid species according to the overall mean availability of aphids (Ives et al. 1999), keeping the vulnerability of aphids constant across a latitudinal gradient (Gagic et al. 2011). Finally, the degree of specialization tended to be constant along the gradient. The highly connected networks found in our study were characterized by low specialization. In winter, it has been demonstrated that parasitoids adopt generalist strategies due to a shortage of optimal hosts (Andrade et al. 2016; Tougeron et al. 2017; Ortiz-Martínez et al. 2019), suggesting that competition among adult parasitoids may be happening along the gradient. The increase in the number of hosts shared by parasitoids in Chile could be related to the possible reduction in host availability (Peñalver-Cruz et al. 2017). However, the efficiency of parasitoids in controlling host pests depends on their genotype and their ability to oviposit and develop on different host species (Raymond et al. 2016), while aphid parasitoid species are known to display different host resource exploitation strategies while exploiting the same host species (van Baaren et al. 2004; Le Lann et al. 2012). Therefore, parasitoids may optimize the efficiency and stability of the biological control service throughout space and time (Raymond et al. 2016).

## Conclusion

Describing variations in food web structure is a critical first step towards improving the understanding of the aphid–parasitoid community composition and its function in agroecosystems along a gradient. Overall, our results suggest no that the temperature does not have a major effect on aphids and parasitoids within these sites and temperature range. Instead, a bottom-up effect was found to be the major driver of the host–parasitoid interactions described here. Our findings are in accordance with the neutral hypothesis, which states that the network structure could simply emerge from random encounters among individuals and is thus solely influenced by the species abundance (Canard et al. 2014). Despite the presence of simple food-web structures along the winter conditions, the cereal crops present in the central-south valley of Chile support a high parasitoid abundance and a relatively large number of species, suggesting that overwintering parasitoid populations/species are not only active but also reproduce. Active foraging of parasitoids during the winter could be an important aspect of their biology that allows them to maintain aphid populations at low densities until spring. Therefore, the use of network-based approaches to understand the relationships between different trophic levels and biological control could yield important insights for pest reduction. Consequently, it will be necessary to follow the whole aphid–parasitoid–hyperparasitoid community in the coming years to determine the success of the biological control of aphids over the long term under fluctuating and changing environmental conditions. This community composition may change rapidly as a result of changes in the spatio-temporal dynamics of biological control as winter conditions may continue to be altered, as observed in other areas, like the west of France, which is experiencing increasing mild winter conditions (Tougeron et al. 2018a, 2020).

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## Authors' contributions

AAT, JVB, BL and CLL conceived the ideas and designed the methodology; AAT, JKAB, and JVB collected the data; AAT, KT and CLL analyzed the data; AAT wrote the first draft. All authors contributed critically to the drafts and gave their final approval for publication.

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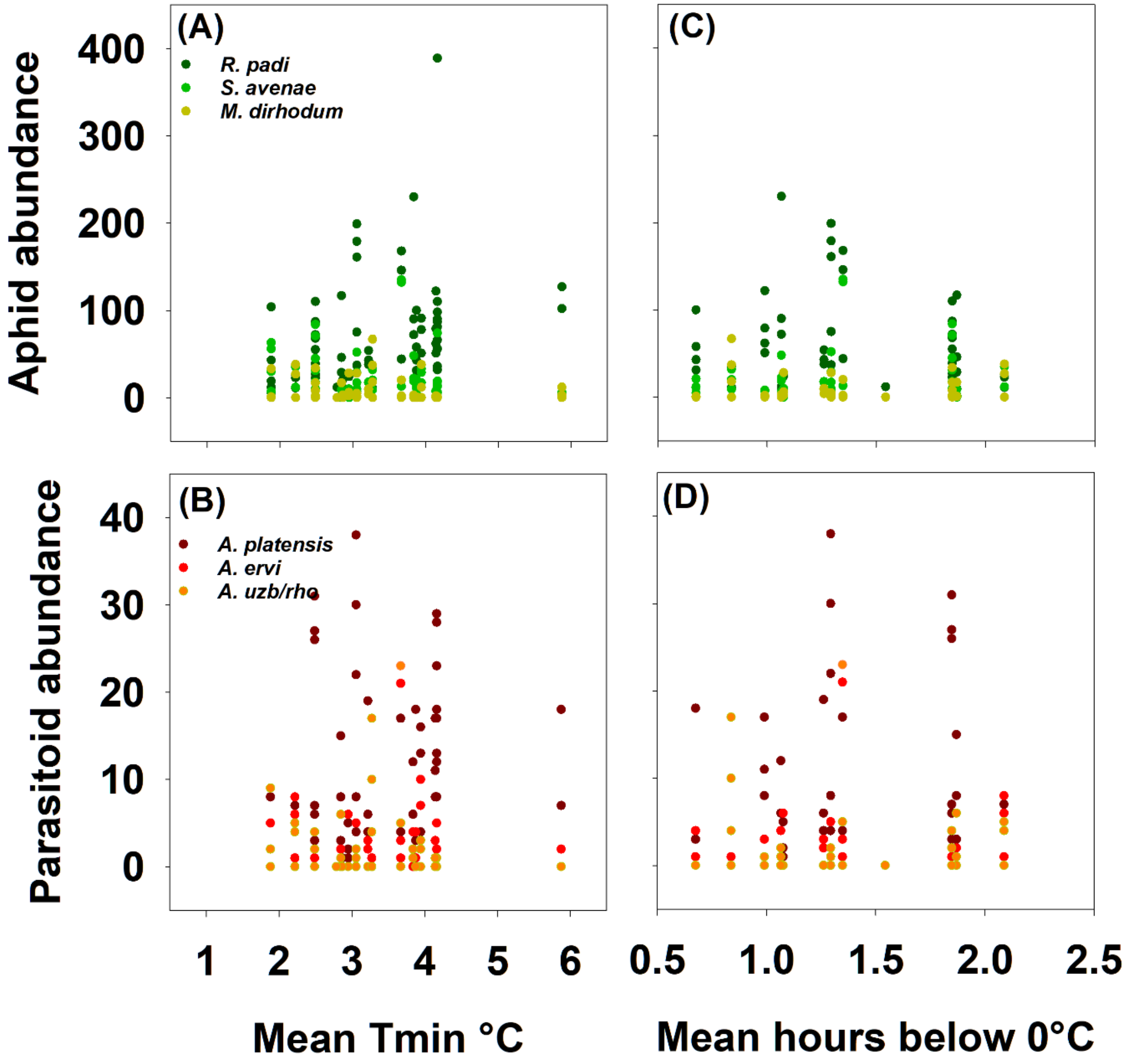
**Appendix 2.1. Figure S2.1.** Climatic data in nine sampled localities and three consecutive years. T mean: Mean temperature (°C), T min: minimal temperatures over winter season and Mean hours < 0°C: Occurrence of temperatures below 0°C over winter season.

| Locality | Year | T mean | T min | Mean hours < 0°C |
|---|---|---|---|---|
| La Serena | 2016 | 11.52 | 5.87 | 0.00 |
| | 2017 | 11.34 | 6.09 | 0.00 |
| | 2018 | 10.80 | 5.49 | 0.01 |
| Rancagua | 2016 | 8.79 | 3.94 | 0.28 |
| | 2017 | 8.75 | 3.30 | 0.32 |
| | 2018 | 9.14 | 2.95 | 1.08 |
| Talca | 2016 | 9.09 | 4.16 | 0.40 |
| | 2017 | 7.85 | 3.05 | 1.29 |
| | 2018 | 7.71 | 2.49 | 1.85 |
| Chillan | 2016 | 9.31 | 4.46 | 0.50 |
| | 2017 | 8.81 | 4.30 | 0.40 |
| | 2018 | 8.41 | 3.67 | 1.35 |
| Pinto | 2016 | 8.56 | 3.76 | 0.70 |
| | 2017 | 8.05 | 3.49 | 0.97 |
| | 2018 | 7.77 | 2.22 | 2.09 |
| Yungay | 2016 | 8.87 | 3.87 | 0.67 |
| | 2017 | 7.95 | 3.22 | 1.26 |
| | 2018 | 7.91 | 2.86 | 1.07 |
| Cunco | 2016 | 7.27 | 1.88 | 2.63 |
| | 2017 | 6.81 | 2.85 | 1.87 |
| | 2018 | 7.12 | 2.78 | 1.54 |
| Temuco | 2016 | 8.12 | 4.02 | 1.01 |
| | 2017 | 7.83 | 4.14 | 0.99 |
| | 2018 | 7.95 | 3.84 | 1.07 |
| Osorno | 2016 | 7.93 | 4.22 | 0.61 |
| | 2017 | 7.14 | 3.67 | 0.74 |
| | 2018 | 7.22 | 3.27 | 0.84 |

**Appendix 2.2. Figure S2.2.** Representation of the relationship between the abundance per field of the main aphid species (*Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum*) and the main parasitoid species (*Aphidius platensis*, *Aphidius ervi* and *Aphidius uzb/rho*) with the temperature variables: (A) Aphids vs Tmin, (B) Parasitoids vs Tmin, (C) Aphids vs hours below 0°C and (D) Parasitoids vs hours below 0°C.



# Part 3

# Seasonality strategies in aphid parasitoids

## Chapter III:
## Overwintering strategies of *Aphidius platensis*

## Chapter IV:
## Diapause in *Aphidius ervi*

# Overwintering strategies and life-history traits of different populations of *Aphidius platensis* along a latitudinal gradient in Chile

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## Abstract

The onset of an overwintering strategy to overcome cold temperatures of a species of ectotherms can include remaining active or entering diapause. This in turn will depend on the relative costs of each strategy and therefore, could differ among populations along a latitudinal gradient. Thus, expecting higher levels of diapause in the coldest conditions and a higher incidence of individuals remaining active in the warmest conditions. We assessed the insect responses to photoperiod and temperature, in five Chilean populations of the aphid parasitoid *Aphidius platensis*. We analyzed the variation in winter temperature along the latitudinal gradient and, under controlled conditions, examining the potential effects of three constant conditions of photoperiod/temperature: 8:16LD (Light: Dark, hours) at 10°C, 10:14LD at 14°C, and 16:8LD at 20°C, on diapause levels. Finally, we measured cold tolerance ($CT_{Min}$), developmental time, fresh body mass and size, fat and water content, and egg load for the emerging parasitoids. Our results showed no clear latitudinal gradient in temperature but differences among sites were clear. None of the five populations of *A. platensis* expressed diapause at any tested condition, suggesting that the environmental thresholds for diapause induction are perhaps not reached in this species under the studied latitudes. Insects from the coldest point in the gradient (Pinto) showed the lowest $CT_{Min}$ suggesting local adaptation. Moreover, physiological, and life-history traits seem to adjust rapidly through developmental thermal acclimation, showing that plasticity is involved in the parasitoid's responses to the temperature differences found among localities. Consequently, both local adaptation and phenotypic plasticity contribute to this species remaining active during the whole winter, being an effective strategy to diapause in relatively mild and stable thermal environments.

**Key words:** Winter; environmental variability; physiology; developmental thermal plasticity; acclimation; local adaptation

## Introduction

Low temperatures are among the most important abiotic factors limiting the range and activity of ectotherms in temperate climates. Survival in winter depends on the magnitude, frequency, and duration of cold events (Bale 2002). Winter conditions are important drivers of individual performance, community composition and ecological interactions (Williams et al. 2015). Changes in photoperiod and temperature are widely used by insects to regulate temporal patterns of development and behaviour, including overwintering strategies (Denlinger et al. 2017). Insects have three options to survive winter conditions: migration, diapause (i.e., arrest of development) or activity to resist the cold (Leather et al. 1995, Bale 2002).

Diapause is a dynamic state of low metabolic activity, genetically determined and neuro-hormonally mediated. It is characterized by behavioral inactivity, morphogenesis and reproductive function arrest, and slowing growth (Denlinger 2002, Tauber et al. 1986). In aphid parasitoids, diapause occurs at a species-specific stage of ontogenesis (larval diapause) and, its expression is mainly regulated by environmental signals like shortening of day length and decrease in temperature and their interaction (token-stimuli), whereas, maternal effects only play a marginal role (Brodeur & McNeil 1989, Polgár & Hardie 2000, Tougeron et al. 2017)

Another strategy employed by some species is to remain active during the winter. Relatively mild winters allow insects to complete their development (Langer & Hance 2000), and reach the threshold of temperature for their activity (Danks 1991). This strategy involves important behavioral and physiological adaptations (Danks 2007) to improve survival in winter conditions (Gibert et al. 2007, Overgaard & Sørensen 2008), in which thermal tolerance plays an important role (Le Lann et al. 2011a). This feature can explain seasonal variations in a species' distribution and abundance (, Tougeron et al. 2016, Andreadis & Athanassiou 2017) as well as variation on the duration of the developmental stages as individuals need a minimum body size to survive these winter conditions (Gotthard 2001). In cold environments, tolerance depends on biochemical and physiological processes (Colinet et al. 2007c). Insects need to accumulate lipid reserves (Sinclair 2015, Sinclair & Marshall 2018,) and increase or decrease their water content according to the species (Lee 1991) in order to have a significant advantage to survive these stressful environments.

Environmental gradients are known to exert a major effect on patterns of intraspecific variation and populations matching their physiology to the local thermal environment (Gaston & Spicer 1998, García-Robledo et al. 2016). Consequently, life-history traits and overwintering strategies covary with latitudinal or altitudinal clines (Hardy et al. 2015). Winter conditions are characterized by shorter days and low temperatures with gradual changes along a given latitudinal gradient. Consequently, for any given year, diapause should occur sooner and more often at high latitudes (harsher winters) than at low latitudes (milder winters) (Zhang et al. 2017). This was demonstrated in different species (e.g.: *Nasonia vitripennis* Walker (Hymenoptera, Pteromalidae)), showing that northern populations (harsher winters) in Europe require longer photoperiods for diapause induction, than southern populations (milder winters), which express more diapause at shorter photoperiods (Paolucci et al. 2013). Equally, ecological conditions may affect the evolution of life-history strategies of a population (Lancaster et al. 2017). As life-history traits coevolve with the physiology of the organism, a single phenotype is formed, which has been referred to as a pace-of-life syndrome (Ricklefs & Wikelski 2002, Moschilla et al. 2019). In winter, physiology and life-history traits may show inter-population variations along a geographic gradient for individuals that remain active. Parasitoids depend on a series of adaptations for survival and are highly susceptible to biotic and abiotic changes (Hance et al. 2007). The geographical origin of a population has a strong influence on their cold tolerance (Colinet & Boivin 2011); this is how individuals from high latitudes tend to be better adapted to colder environments than those from lower latitudes (Hoffmann et al. 2002, David et al. 2003). In addition, a negative relationship between body size and developmental temperature, favouring larger organisms developing in colder conditions, a phenomenon known as the `Temperature-Size Rule` has been described for a same species (Atkinson 1994, Angilletta & Dunham 2003). Moreover, life-history traits, that are often positively correlated all together, such as developmental time (Zamani et al. 2007), fat content (Arrese & Soulages 2010, Foray et al. 2013), survival (Colinet et al. 2006), early fecundity and body size (Le Lann et al. 2011b, Horne et al. 2018) increase with decreasing temperatures, which is developmental plasticity. Finally, cold tolerance is also highly plastic to thermal conditions experienced prior to cold stress (Foray et al. 2013), and depends on both life-history timing and seasonal temperature profiles (Le Lann et al., 2011a; van Baaren et al., 2010). Therefore, life-history traits may be correlated with temperature along the latitudinal gradient and influences physiological traits such as cold tolerance (Addo-Bediako et al. 2000).

In this study, we compared the response of five populations of *Aphidius platensis* Brethes (Hymenoptera, Braconidae) distributed along a latitudinal gradient in the central-south valley of Chile, to constant regimens of temperature and photoperiod simulating winter, fall and spring conditions. We measured diapause levels as well as physiological and life-history traits of active (non-diapausing) individuals. In this region, *Rhopalosiphum padi* (Linnaeus), *Sitobion avenae* (Fabricius) and *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae) are the predominant aphid species attacking cereal crops. Their natural enemy guild is composed mainly by the Aphidiinae group, primarily the *Aphidius* genus (Starý 1995) and *A. platensis* is a common parasitoid species reported in Chile to use *R. padi* species as hosts (Alfaro-Tapia et al., *in preparation*). It is the most abundant and prevalent species in winter, along a gradient of 595 km with relatively mild winter temperatures but with clear differences in temperature due to a North-South latitudinal change (Alfaro-Tapia et al., *in preparation*). Therefore, the following hypotheses were tested: (1) *A. platensis* expresses low diapause levels, (2) if diapause is expressed, then its level should be higher in parasitoid populations from cold localities than from mild ones, (3) for individuals that remain active during the winter, developmental time should be longer and with larger body size in populations from cold latitudes according to the temperature-size rule, (4) adults from cold latitudes should be more cold-resistant than those from mild environments, affecting numerous other life-history traits, (5) since mass/size are positively correlated with fat reserve content (Colinet et al. 2007a, b), we expect that individuals from colder latitudes that develop in winter conditions, will have a higher fat content, and (6), they should display higher egg-load and produce larger eggs.

# Material and methods

## Meteorological data and gradient determination

During the winter of 2018, we obtained environmental values from meteorological stations (https://www.agromet.cl/), nearest to each of the five localities where *A. platensis* populations were collected along a latitudinal gradient of 595 km in the central-south valley of Chile: Rancagua (34°09′S, 70°44′W and 469 m altitude), Talca (35°22'S, 71°50'W and 189 m), Chillan (36°32′S, 72°04′W and 154 m), Pinto (36°41'S, 71°46'W and 519 m) and Temuco (38°55'S, 72°02'W and 114 m) (Figure 3.1 A). We evaluated the following environmental variables at each locality: minimum daily temperature (Tmin), maximum daily temperature (Tmax), mean daily temperature (mean), and the total number of hours (h) with temperatures below zero, which is close to freezing temperature and below the developmental thresholds of most *Aphidius* species (Sigsgaard 2000). We chose these variables because they have a great influence on populations' persistence during winter (Honek et al. 2018) and may be good predictors of diapause level (Tougeron et al. 2018a). The central-south valley of Chile is a region along the west coast of subtropical South America, limited to the east by the Andes (Garreaud 2013) and to the west by the coastal mountain range. Although, as a consequence of climate change, inter-annual homogenization of temperatures has affected latitudinal gradients everywhere (Falvey & Garreaud, 2009) consistent temperature differences among localities can be observed (Burger et al. 2018). This area encompasses highly productive cereal crops in a temperature gradient, with mean temperatures ranging between 8.4 and 9.1°C, mean minimum temperatures ranging from 2.6 to 5.0°C, and mean maximum temperatures ranging between 12.2 and 13.6°C during winter (del Pozo & del Canto, 1999).

## Insect sampling and rearing

Aphid mummies of the five populations were collected from cereal crops, between July 7th and August 22th of 2018, along the above described latitudinal gradient. From north to south, we sampled one field in Rancagua, seven fields in Talca, two fields in Chillan, two in

Pinto and four in Temuco. A minimum of 32 and a maximum of 167 mummies were collected from each field. Mummies collected were isolated individually in 1.5 mL Eppendorf tubes and kept in laboratory conditions at 20±1°C, 65±5 % RH, until emergence. Emerging parasitoids were identified at the species level using taxonomic keys (Starý 1995, Tomanović et al. 2014). Initially between 20 to 60 individuals of parasitoids identified as *A. platensis* were used to establish a mass rearing population on a polyclonal *R. padi*. The host population was collected prior to the establishment from cereal crops in the winter of 2018 from the central-south valley of Chile. Aphids were reared on potted wheat *Triticum aestivum* (Linnaeus) in Plexiglas cages of 0.40 $m^3$ and maintained at laboratory conditions at 20±1°C, 65±5% RH and photoperiod of 16:8 LD. After the establishment of the mass rearing population, species confirmation was made by molecular approaches (Tomanović et al. 2014). Finally, to prevent differential adaptation of parasitoids populations to the aphid strain, another polyclonal population of *R. padi* collected in Brittany (France) was used and maintained under the same conditions mentioned above. This aphid strain was highly suitable to parasitism, confirmed by the number of total mummies formed and the emergence rate (~90%) regardless of the tested conditions.

## Experimental conditions

To evaluate the effects of photoperiod and temperature on diapause incidence of the five populations described above parasitized aphids coming from each population were distributed among three different conditions: (1) winter condition: 8:16 LD (Light: Dark, h) at 10°C, (2) fall: 10:14 LD at 14°C, (3) spring condition: 16:8 LD at 20°C. These conditions were chosen for the following reasons. (1) Winter condition: at this temperature and photoperiod, *Aphidius* species from temperate climates are induced to diapause maintaining this state until spring (Langer & Hance 2000, Polgár & Hardie 2000, Tougeron et al. 2017). (2) Fall condition: parasitoids usually enter diapause in the fall, with photoperiod and temperature as influential abiotic factors (Brodeur & McNeil 1989). (3) Spring condition: this condition does not induce diapause in populations from temperate climates (Tougeron et al. 2017).

For the experiments, individual mummies from the mass rearing of each population were isolated from plants in gelatin capsules and checked for emergence twice each day. Emerging parasitoids were sexed and mated. To ensure mating, five newly emerged females were placed with two to three males of the same age in micro-cages (L=10 cm, Ø=5 cm) for 24 h with a diluted honey solution in water (30%) to feed them. Subsequently, these five mated females were introduced to cages of 30 $cm^3$ containing one wheat pot with 150±10 parthenogenetic synchronized *R. padi* aphids between second and third instar (Brodeur & McNeil 1989) at spring-like conditions. Honey solution and water were placed on cotton feeders to allow the parasitoids to survive. All females used in the experiments were less than two days old and naive in terms of oviposition experience. After 24 h, female parasitoids were removed from the cages and the 150±10 potentially parasitized aphids were randomly distributed on six wheat pots (≈25 aphids per pot). Pots consisted of plastic tubes (L=16 cm, Ø5 cm) filled with vermiculite substrate and were closed using 100% nylon white organdy mesh. Finally, two pots were placed in each of the three conditions. Each pair of tubes was considered as one replica. The experiment was repeated until there were at least 200 mummies for each condition per population. All the experiments were conducted within seven months after the establishment of the parasitoid populations.

## Measure of the diapause level

To evaluate the diapause level in each condition for each population, parasitized aphids were incubated in climate chambers (Sanyo, Osaka, Japan) under the three experimental photoperiod/temperature conditions described above. After three days, incubated aphids were checked daily until mummification. One-day-old mummies were individually confined in gelatin capsules. The aphids which had not become mummies within three days after the last mummification were considered as unparasitized. The collected mummies were checked daily for parasitoid emergence. Fifteen days after the last adult emergence, all the non-emerged mummies of each condition were dissected as in Tougeron et al. (2017). The dissections were performed under a stereoscopic microscope (65x Nikon SMZ800) linked to a video camera (JVC KY-F50) by carefully piercing a tiny hole in the mummy envelope without damaging the individual inside. In this way their state (dead immatures/adults or diapausing golden-yellow plump pre-pupa) could be accurately assessed following (Tougeron et al. 2017).

## Measures of physiological and life-history traits

Since only non-diapausing individuals were obtained for the five populations in the three conditions, the following physiological and life-history traits were measured: At emergence, the cold tolerance of 750 parasitoids of the different tested conditions was measured using critical minimal temperatures ($CT_{Min}$) as they are strongly linked to insect performance (Huey 2010). Other parasitoids were frozen at -20°C until further measurements. One batch of these parasitoids was used to measure water content (WC) and fat content (FC), where different parameters were calculated (dry mass DM, water mass WM, water content WC, lean dry mass LDM, fat mass FM and fat content FC). It has been demonstrated that these measurements affect the response of parasitoids to cold tolerance (Colinet et al. 2006, Hance et al. 2007, Colinet & Hance 2010). In addition, a second batch of females was used to evaluate the egg load at emergence and the mean volume of eggs. Finally, fresh body mass (FBM) and tibia length were measured. These measures are good indicators of fitness in parasitoid wasps and may be used as primary proxies (Roitberg et al. 2001). Many traits of parasitoids, including thermal tolerance (Le Lann et al. 2011a), WC and FC (Ismail et al. 2010) and fecundity (Opp & Luck 1986, Rosenheim & Rosen 1991, Roitberg et al. 2001) are positively correlated with body mass and size.

### *Fresh body mass and hind tibia length measurements*

FBM was measured with a microbalance Mettler Toledo®-XP6U-Mass Comparator (Me22; sensitivity: 1µg) for 75 females and 50 males of each population per condition. The right metathoracic tibia was measured using a camera connected to a stereoscopic microscope (65X of total magnification) using the ImageJ software (Abràmoff et al. 2004).

### *Critical thermal minima (CTMin)*

To assess cold tolerance in *A. platensis*, 25 females and 25 males that emerged from each condition and from the five different populations were individually transferred to a thin-walled glass tube (35 x 5 cm) adapted from Powell & Bale (2006) and topped with a cotton plug to maintain a stable internal environment and a constant relative humidity at 50±10% (Le Lann et al. 2011a). Ethylene glycol from a thermostat bath (Lauda RE320, Lauda-Königshofen, Germany) controlled the air temperature in the inner chamber of the column to cool the tube. The temperature in the inner chamber was recorded using a thermal probe (sensitivity of 0.1°C) (Comark C8600 TempScan Digital Bench Lab Scanning Thermometer). Prior to the beginning of the experiment, parasitoids with no more than two hours of emergence, were allowed to acclimate in the glass tube for 15 minutes at 20°C. Parasitoids were then cooled from this temperature to the last critical limit at a rate of 0.75°C min$^{-1}$; this temperature was chosen to avoid inducing a fast cold hardening response (Powell & Bale 2006). Two types of critical minimal limits ($CT_{Min1}$ and $CT_{Min2}$) were recorded as recommended in Terblanche et al. (2007) and, Le Lann et al. (2011a). The critical thermal minimum was evaluated as both 'locomotion trouble' when parasitoids were still able to walk but without coordination, corresponding to the loss of coordinated muscle functions ($CT_{Min1}$), and 'total paralysis' when parasitoids become completely immobile and no appendage movements are visible. At this temperature, individuals lose their ability to adhere to the wall of the tube and therefore fall, which is referred to as 'chill coma' ($CT_{Min2}$) (Le Lann et al. 2011a), a reversible state highly correlated with the recovery time (Andersen et al. 2015). After both $CT_{Min}$ measurements, parasitoids recovered from the cooling process, were placed individually in 0.2 mL Eppendorf tubes and stored in the freezer at -20°C. Subsequently, FBM was measured as it can influence $CT_{Min}$ responses (Le Lann et al. 2011a).

### *Water and fat content*

To evaluate the WC and FC, we used the method described by Ismail et al. (2010). Twenty-five females and twenty-five males emerged from each population and condition were stored at -20°C and measured. The FBM was obtained by weighing the individuals as described above. Parasitoids were then placed into an air oven at 60°C for three days and reweighed to obtain DM. WM was obtained as WM=FBM−DM and WC was calculated as a ratio between the WM and the DM (WC=WM/DM) (Košťál et al. 2004). To evaluate the FC, each dried individual was placed for two weeks in a 1.5 mL Eppendorf tube containing 1 mL of the extracting solution based on chloroform: methanol (2:1) at room temperature under a fume hood. Parasitoids were then dried for 12 h in an air oven at 60°C to remove the extracting solution in order to measure the LDM. FM was obtained by subtracting LDM from DM, and finally the FC was calculated as: FC= FM/LDM. The FC is a good proxy of fitness as it can be used to characterize the costs associated with the maintenance and development in insects (Sinclair 2015, Sinclair & Marshall 2018).

### *Measure of egg load and volume of the eggs*

The egg load and volume of the eggs were measured in twenty-five females stored at -20°C for each population and condition. After being weighed and having the tibia length measured, each female was placed in 50 µL of distilled water on a microscope slide and dissected under a stereoscopic microscope (65x Nikon SMZ800). All mature eggs were counted and photographed using a camera (Sony N50) mounted on the microscope. The length and width of 20 randomly chosen mature eggs from each female were measured with the numeric image analysis software ImageJ (Abràmoff et al. 2004) in order to calculate the volume of the eggs using the following formula: $V = (2/3) * \prod(L/2*(w/2)^2)$, where, $L$=length, $w$=width, as in Le Lann et al. (2011b).

## Statistical analyses

### *Gradient determination*

For our analysis, we performed a non-metric multidimensional scaling (NMDS) analysis to group localities by climatic similarities based on a distance matrix following a Principal Component Analysis (PCA) representation. Then, we used the minimum temperature (positively correlated with mean and maximum temperatures) and the number of hours with temperatures below zero (negatively correlated with the mean, maximum and minimum temperatures) for the statistical analyses. We used repeated-measures ANOVA. Localities were considered as a fixed effect, and the day was included as a random effect. Tukey HSD post hoc tests were used to detect differences among localities. For a better understanding of the gradient, mild and cold localities were combined in the tables and figures, although they were analyzed separately.

### *Parameters measured*

Statistical models were fitted to the data to observe interactive effects of the population origin (five levels), and the tested conditions (three levels) on physiological and life-history traits. According to the structure of our data, a survival logistic-rank model (LR) (survival package) and generalized linear models (GLMs) were built. Although the sex of the parasitoids was not part of our predictions, we included it in our analyses as an additional fixed factor because many traits vary between males and females (Ismail et al. 2010, Le Lann et al. 2011a). Only significant interactions are shown in the results. For the full models run, see supplementary material (Appendix 3.1, Table S 3.1). In addition, to observe if there was an effect of body size/mass, we used tibia length or FBM as a covariate. The best model structure was determined by stepwise removal of insignificant higher order interactions by using Akaike information criterion and performing an ANOVA type II from the *car* package (Fox et al. 2016). Pairwise comparisons were done using 'Tukey tests', correcting for multiple comparisons by the 'single-step' method using *Multcomp* package (Hothorn et al. 2017). All statistical analyses were conducted with R 3.6.5 statistical software (R Core Team 2019).

Since no diapausing individuals were obtained, we used GLM models in order to compare the emergence rate and dead larvae/adults, assuming a binomial distribution and logit link functions. The emergence rate was estimated as the mean proportion of parasitoids which emerged from the formed mummies (Henry et al. 2010), and non-emerged mummies were dissected to determine parasitoids stage. Additionally, a survival logistic-rank model was used adding a censoring factor for non-emerged mummies to evaluate the total developmental time. Models were compared using a likelihood-ratio chi-square method.

FBM, hind tibia length, CTLs ($CT_{Min1}$ and $CT_{Min2}$), WC and FC data were analysed using a GLM assuming a Gaussian distribution and 'identity' link function to compare differences between populations, conditions and sexes. In the case of CTLs, FBM was incorporated as a covariate and global correlations were performed using Pearson correlation tests on individuals emerged from the three conditions between FBM and $CT_{Min1}$ and $CT_{Min2}$. (Le Lann et al. 2011b). To establish differences in WC and FC, the tibia length was used as a covariate. Parameters measured to calculate the WC and FC (i.e., FBM, DM, WM, LDM and FM) were not used in the statistical analysis as these are highly correlated (see Appendix 3.2, Figure S 3.2). Pearson correlation tests were performed on FBM and hind tibia length. Finally, the effect of the populations and environmental conditions on the egg load and volume of the eggs were analysed using a GLM model. As the Poisson model exhibited overdispersion a negative binomial distribution was used. A gamma error was used to compare the volume of the eggs. In both cases, tibia length was used as a covariate.

## Results

### *Meteorological data and gradient determination*

According to the NMDS analyses, Rancagua, Chillan and Temuco can be grouped together as 'mild winter localities' and Talca and Pinto as 'cold winter localities' supported by 0.20 stress value. The PCA representation shows that coldest winter localities were characterized by more hours below zero and mild winter localities by higher mean minimum temperatures (Figure 3.1 B). Repeated measures ANOVA showed differences among localities of the mean minimum temperatures (*df*=4; F=11.35, $P<0.001$) and the mean numbers of hours with temperatures below zero (*df*=4; F=4.95, $P<0.001$). For both environmental variables, Pinto and Talca were colder than Rancagua, Chillan and Temuco localities (Figure 3.1 C).

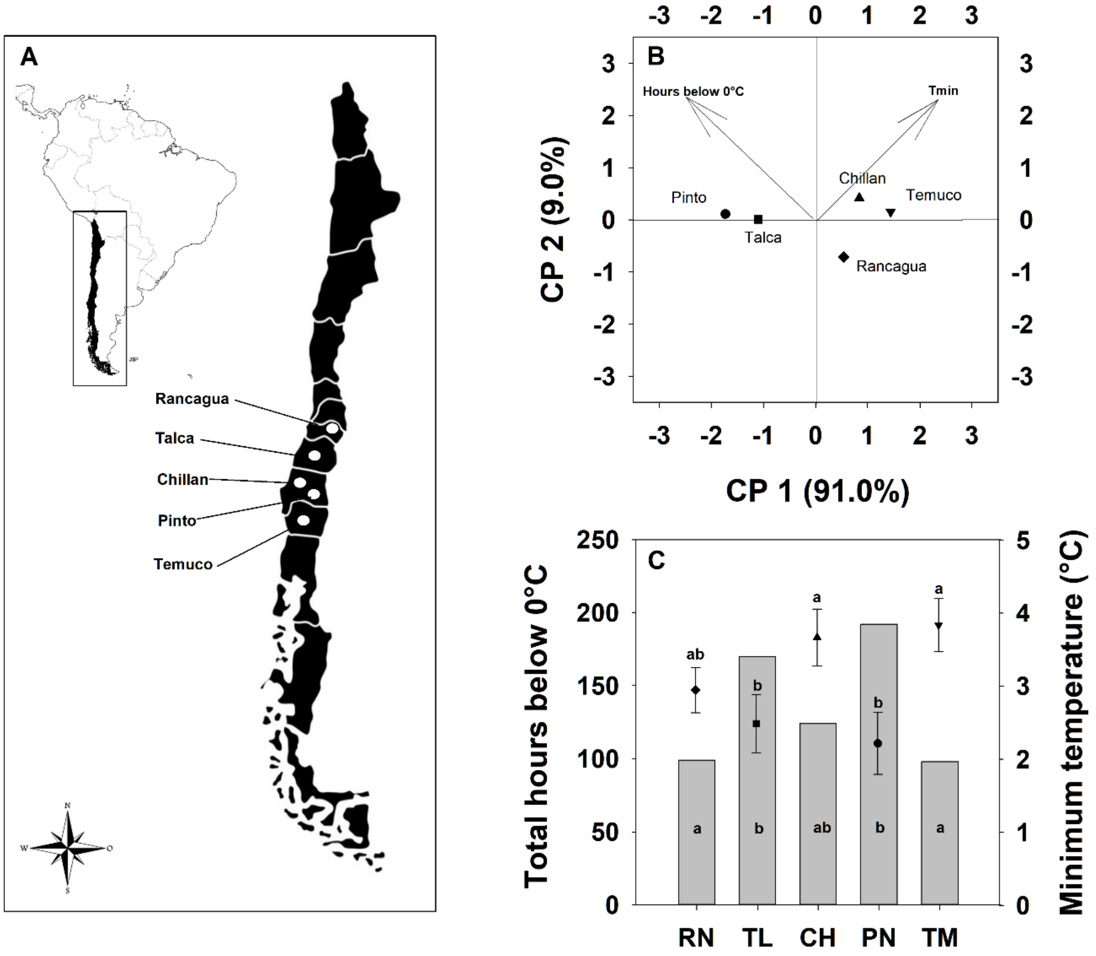

**Figure 3.1 .** Geographic and environmental gradient along the central south valley of Chile in winter 2018: (A) Study sites, (B) Biplot of the principal component analysis (PCA) for environmental variable in the meteorological stations near to the sampling localities. The PC1 and PC2 shows clear partitioning according to minimal temperatures 'Tmin' and numbers of hours below 0°C; (C) Total hours below 0°C (grey bars) and mean ± SE of minimum temperature (dots) for each sampled latitudinal locality (RN: Rancagua, TL: Talca, CH: Chillan, PN: Pinto and TM: Temuco).

### *Emergence rate, diapause expression, and developmental time*

A total of 3,691 mummies were formed in laboratory induction tests. Overall, parasitoid emergences for each population were not reduced by low temperatures and a short photoperiod (Table 1). The emergence rate was approximately 90% for all populations (GLM:

$\chi^2$ =0.69; *df*=4, *P*=0.95) and for all conditions (GLM $\chi^2$=0.42, *df*=2, *P*=0.81), without interaction between these factors. The dissection of the non-emerged mummies showed around 80% of fully formed adults and 20% of dead grey/dark adults and larvae (Table 3.1) among populations (GLM: $\chi^2$=0.01; *df*=4, *P*=1.00) and conditions (GLM: $\chi^2$ =0.01, *df*=2, *P*=0.99).

Total developmental time was significantly different with respect to populations (LR: $\chi^2$ =35.9; *df*=4, *P*<0.001) and conditions (LR: $\chi^2$=12,590.0; *df*=2, *P*<0.001), where individuals took an average of 13.30±0.03 (mean ± SE), 22.31±0.04, and 38.64±0.06 days to emerge from spring, fall and winter conditions, respectively. Also, an interaction between population $^x$ condition (LR: $\chi^2$=12,590.0; *df*=2, *P*<0.001) was observed. This was mainly explained by the slightly faster emergence of Pinto (TukeyHSD, *z*=-82.59, *P*<0.001) and Chillan populations (TukeyHSD, *z*=3.72, *P*<0.001) when compared to the Temuco population under spring conditions, whereas individuals form the Pinto populations had a slower emergence (TukeyHSD, *z*=5.11, *P*<0.001) than those from Chillan under winter conditions (Figure 3.2).

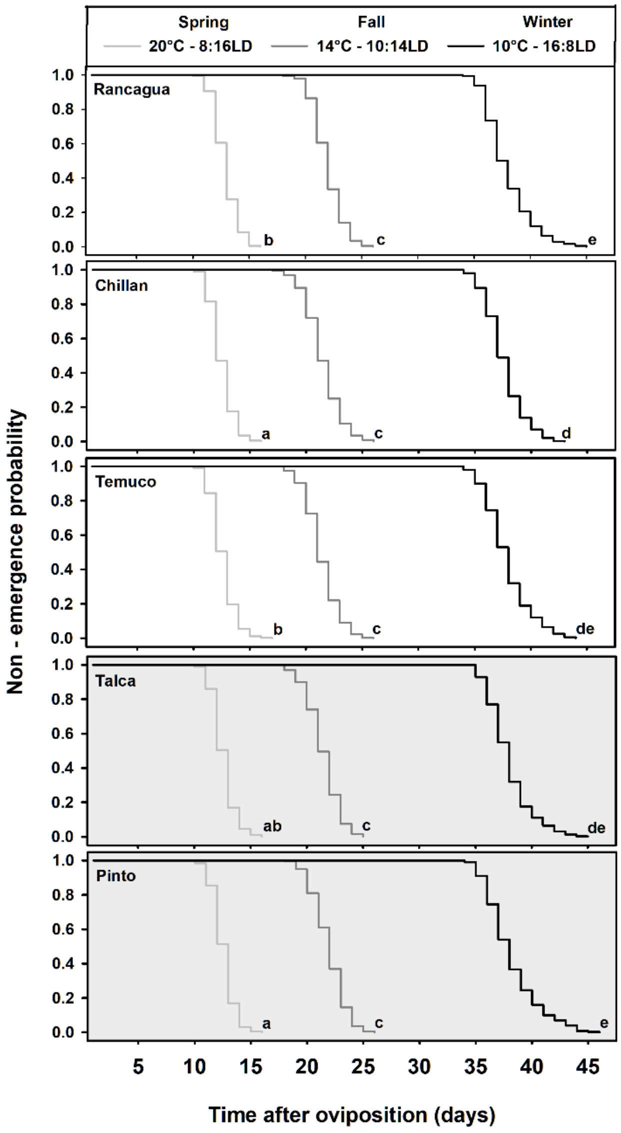

**Figure 3.2.** Probability of emergence in days since oviposition to emergence of *A. platensis* from five populations exposed to three conditions of temperatures and photoperiod (spring, fall and winter conditions). Different letters indicate a significant difference between populations and conditions. Tukey HSD post hoc test ($P < 0.05$). The white part shows the mild winter localities and grey parts the cold winter ones.

**Table 3.1.** Results of exposure of aphids parasitized by five populations of *A. platensis* in three conditions of temperatures and photoperiod: Emergence rate, N° of mummies formed, N° of non-emerged mummies dissected 15 days after the last emergence and Proportion of dissected non-emerged mummies containing a dead adult the remaining proportion corresponds to dead larvae. No mummies in diapause were found. Mean ± SE are shown. The white part shows the mild winter localities and grey parts the cold winter ones.

| | | Population | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Condition** | **Rancagua** | **Chillan** | **Temuco** | **Talca** | **Pinto** |
| **Emergence rate** | Spring<br>20°C<br>16:08 LD | 0.87<br>(±0.02) | 0.95<br>(±0.01) | 0.90<br>(±0.02) | 0.88<br>(±0.03) | 0.92<br>(±0.02) |
| | Fall<br>14°C<br>10:14 LD | 0.93<br>(±0.02) | 0.94<br>(±0.02) | 0.95<br>(± 0.02) | 0.93<br>(±0.02) | 0.97<br>(± 0.01) |
| | Winter<br>10°C<br>08:16 LD | 0.90<br>(± 0.02) | 0.92<br>(±0.02) | 0.92<br>(± 0.02) | 0.91<br>(± 0.01) | 0.94<br>(± 0.02) |
| **N° of formed mummies** | Spring<br>20°C<br>16:08 LD | 180 | 260 | 247 | 244 | 207 |
| | Fall<br>14°C<br>10:14 LD | 217 | 319 | 232 | 278 | 259 |
| | Winter<br>10°C<br>08:16 LD | 206 | 300 | 209 | 273 | 260 |
| **N° non-emerged mummies** | Spring<br>20°C<br>16:08 LD | 24 | 12 | 24 | 29 | 17 |
| | Fall<br>14°C<br>10:14 LD | 18 | 20 | 11 | 21 | 7 |
| | Winter<br>10°C<br>08:16 LD | 21 | 23 | 18 | 25 | 16 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Proportion of dead adults** | Spring<br>20°C<br>16:08 LD | 0.91 (±0.05) | 0.81 (±0.11) | 0.85 (±0.10) | 0.87 (±0.05) | 0.94 (±0.04) |
| | Fall<br>14°C<br>10:14 LD | 0.88 (±0.05) | 0.83 (±0.10) | 0.86 (±0.11) | 0.90 (±0.05) | 0.93 (± 0.06) |
| | Winter<br>10°C<br>08:16 LD | 0.82 (±0.10) | 0.90 (±0.05) | 0.78 (±0.11) | 0.81 (±0.10) | 0.83 (±0.11) |

### *Fresh body mass (FBM) and size*

There were no differences in FBM between the five populations (GLM: $\chi^2$=1.02; *df*=4, *P*=0.91). However, parasitoids were affected by the photoperiod-temperature conditions (GLM: $\chi^2$=1,705.46; *df*=2, *P*<0.001). The FBM increased significantly with the decrease in temperature and photoperiod. Moreover, females were heavier than males (GLM: $\chi^2$=41.89; *df*=1, *P*<0.001), and there was an interaction effect of population $^{x}$ condition (GLM: $\chi^2$=21.10; *df*=8, *P*<0.05), where the emerged individuals from the Pinto population under fall conditions were slightly less heavy than the Chillan population, but similar to Talca, Rancagua, and Temuco (Table 3.2). A significant effect was observed for the parasitoid populations on the hind tibia length (GLM: $\chi^2$=16.45; *df*=4, *P*<0.001) with a greater size of the parasitoids from Pinto compared to those from Chillan. However, similar to FBM, when temperature and photoperiod conditions decreased, emerging individuals were larger than those from the other conditions (GLM: $\chi^2$=689.22; *df*=2, *P*<0.001). Likewise, females were larger than males (GLM: $\chi^2$=70.56; *df*=1, *P*<0.001) (Table 2.2). In addition, both traits (FBM and size) were correlated positively each other (*R*=0.85, *P*<0.001). (See Appendix 3.3, Figure S 3.3).

**Table 3. 2.** Influence of different conditions on fresh body mass (mg) and hind tibia length (mm) at emergence (N = 50 ♂ and N = 75 ♀) of five populations of *A. platensis* from central south valley of Chile. Mean ± SE are shown. The shaded part shows the cold winter localities and the white part the mild winter localities.

| | | Population | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Condition** | **Rancagua** | | **Chillan** | | **Temuco** | | **Talca** | | **Pinto** | |
| | | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| **Fresh body mass (mg)** | Spring 20°C 16:08 LD | 0.07 (±0.01) | 0.07 (±0.01) | 0.06 (±0.01) | 0.07 (±0.01) | 0.06 (±0.01) | 0.07 (±0.02) | 0.07 (±0.01) | 0.07 (±0.02) | 0.06 (±0.01) | 0.07 (±0.02) |
| | Fall 14°C 10:14 LD | 0.13 (±0.03) | 0.14 (±0.04) | 0.14 (±0.04) | 0.15 (±0.04) | 0.13 (±0.03) | 0.15 (±0.04) | 0.12 (±0.03) | 0.14 (±0.04) | 0.12 (±0.03) | 0.13 (±0.04) |
| | Winter 10°C 08:16 LD | 0.14 (±0.03) | 0.15 (±0.04) | 0.13 (±0.04) | 0.15 (±0.04) | 0.14 (±0.05) | 0.15 (±0.04) | 0.14 (±0.04) | 0.15 (±0.04) | 0.14 (±0.04) | 0.16 (±0.05) |
| **Tibia length (mm)** | Spring 20°C 16:08 LD | 0.48 (±0.02) | 0.50 (±0.02) | 0.48 (±0.01) | 0.49 (±0.01) | 0.48 (±0.01) | 0.50 (±0.01) | 0.48 (±0.01) | 0.51 (±0.01) | 0.49 (±0.01) | 0.51 (±0.01) |
| | Fall 14°C 10:14 LD | 0.53 (±0.01) | 0.56 (±0.01) | 0.53 (±0.01) | 0.56 (±0.01) | 0.54 (±0.01) | 0.56 (±0.01) | 0.54 (±0.01) | 0.56 (±0.01) | 0.54 (±0.01) | 0.57 (±0.01) |
| | Winter 10°C 08:16 LD | 0.56 (±0.01) | 0.59 (±0.01) | 0.56 (±0.01) | 0.57 (±0.01) | 0.58 (±0.01) | 0.59 (±0.01) | 0.56 (±0.01) | 0.59 (±0.01) | 0.57 (±0.01) | 0.60 (±0.01) |

### *Critical thermal limits*

There was a significant effect of the population on $CT_{Min1}$ (GLM: $\chi^2=9.46$; *df*=4, *P*=0.05) and the $CT_{Min2}$ (GLM: $\chi^2=308.43$; *df*=4, $P<0.001$), individuals from the Pinto population were the most cold tolerant. Condition significantly influenced the $CT_{Min1}$ (GLM: $\chi^2=77.05$; *df*=2, $P<0.001$) and the $CT_{Min2}$ (GLM: $\chi^2=1{,}618.56$; *df*=2, $P<0.001$): individuals that emerged under the winter condition had a lower $CT_{Min1}$ and $CT_{Min2}$ than parasitoids that emerged under the two other conditions. Furthermore, there was an effect of sex: $CT_{Min1}$ (GLM: $\chi^2=11.86$; *df*=1, $P<0.001$) and $CT_{Min2}$ (GLM: $\chi^2=176.78$; *df*=1, $P<0.001$), females were more cold tolerant than males (Figure 3.3 A and Figure 3.3 B). In addition, heavier individuals of both sexes had lower $CT_{Min1}$ and $CT_{Min2}$. Average $CT_{Min2}$ for parasitoids assayed from Pinto were lower than those for individuals from other populations under the winter condition. Females from the Pinto population were more resistant to low temperatures under all conditions, as there was a highly significant population $^{x}$ condition (GLM: $\chi^2=27.88$; *df*=8, $P<0.001$) and population $^{x}$ sex interaction (GLM: $\chi^2=10.79$; *df*=4, $P<0.05$). Finally, there was an effect of the FBM on the response of *A. platensis* to $CT_{Min1}$ (GLM: $\chi^2=6.42$; *df*=1, $P<0.05$) and $CT_{Min2}$, (GLM: $\chi^2=727.93$; *df*=1, $P<0.001$) with a strong positive correlation between FBM with $CT_{Min1}$ (Figure 3.3. C) and $CT_{Min2}$ (Figure 3.3 D).

### *Water and fat content*

There was a significant difference in WC among populations with a slight increase for the individuals from Chillan and Rancagua populations compared to those from the Pinto population, although similar when compared to the Talca and Temuco populations (GLM: $\chi^2=22.11$; *df*=4, $P<0.001$). A significant increase was observed when individuals developed under winter conditions (GLM: $\chi^2=110.04$; *df*=2, $P<0.001$). In the same way, females had a higher WC than males (GLM: $\chi^2=7.22$; *df*=1, $P<0.05$), and interaction between populations $^{x}$ condition was observed (GLM: $\chi^2=17.41$; *df*=8, $P<0.05$), where WC from Pinto decreased slightly under spring and fall conditions when compared to the other combinations (Table 3.3). Additionally, larger individuals had a higher WC (GLM: $\chi^2=282.69$; *df*=1, $P<0.001$). The FC was significantly higher under cold winter populations (Pinto and Talca) compared to the other three populations (GLM: $\chi^2$=32.17; *df*=4, *P*<0.001). Likewise, FC increased when temperatures and photoperiod decreased (GLM: $\chi^2$=11.94; *df*=2, *P*<0.001), and larger individuals had a higher FC (GLM: $\chi^2$=30.69; *df*=1, *P*<0.001) (Table 3).

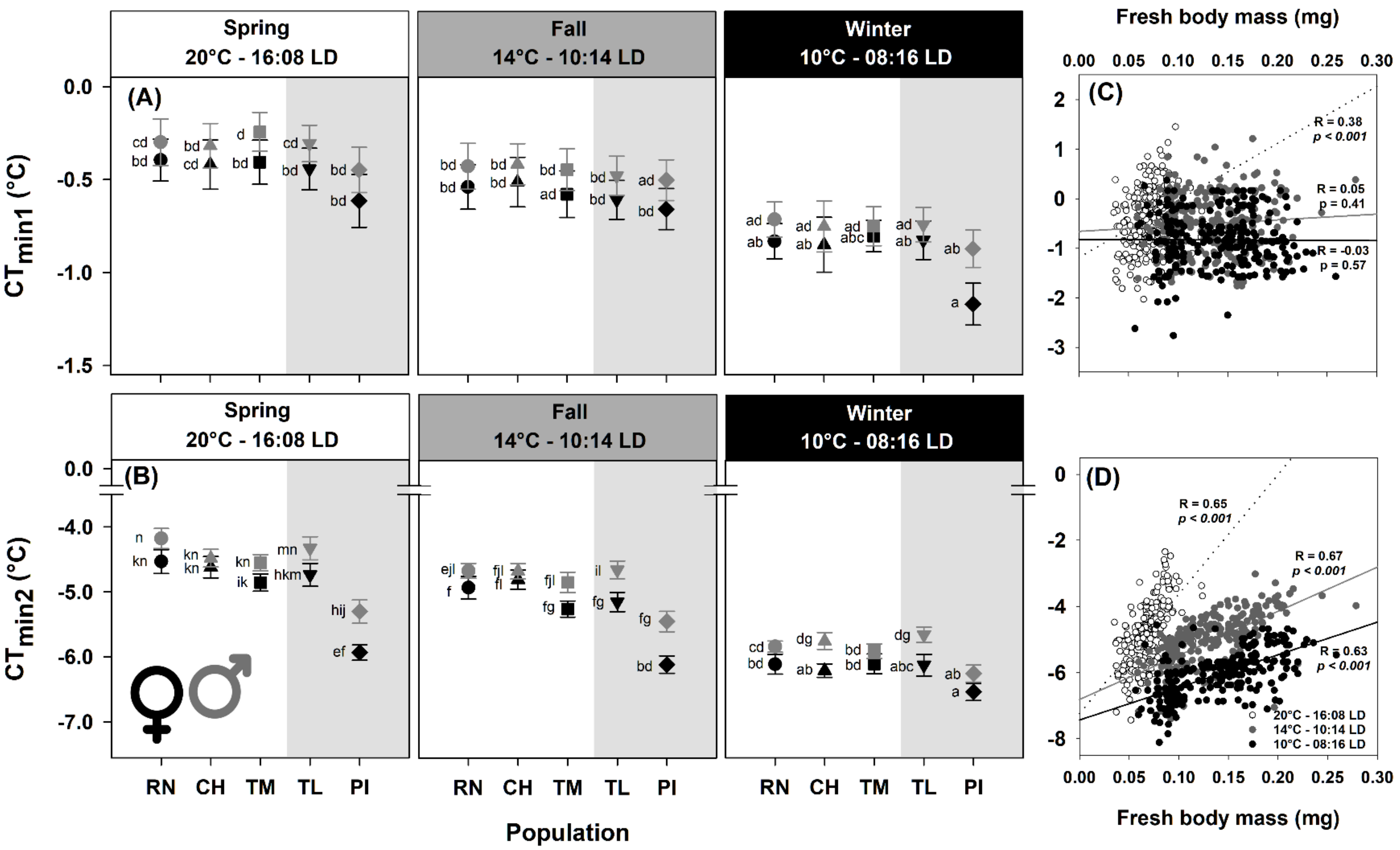

**Figure 3.3.** Critical minimal temperatures ($CT_{Min}$) of males and females of five populations (RN: Rancagua, CH: Chillan, TM: Temuco, TL: Talca and PI: Pinto) of A. platensis exposed to three different conditions: (A) CT of loss of muscular function ($CT_{Min1}$); (B) Fresh body mass of all tested individuals, correlated with the $CT_{Min1}$, (C) CT of chill coma ($CT_{Min2}$), and (D) Fresh body mass of all tested individuals correlated with $CT_{Min2}$. Plots show the mean ± SE. Different letters indicate a significant difference between populations of *A. platensis*, conditions and sexes. Tukey HSD post hoc tests (P < 0.05). The white part shows the mild winter localities and grey parts the cold winter ones

**Table 3.3**. Values of water content (WC) and fat content (FC) of A. platensis from different populations of the central south valley of Chile tested in three different conditions. Mean ± SE are shown. The shaded part shows the cold winter localities and the white part the mild winter localities

| | | Population | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Condition** | **Rancagua** ♂ | ♀ | **Chillan** ♂ | ♀ | **Temuco** ♂ | ♀ | **Talca** ♂ | ♀ | **Pinto** ♂ | ♀ |
| **Water content WC** | Spring 20°C 16:08LD | 0.31 (±0.06) | 0.20 (±0.02) | 0.26 (±0.04) | 0.23 (±0.04) | 0.21 (±0.02) | 0.23 (±0.03) | 0.27 (±0.03) | 0.28 (±0.05) | 0.20 (±0.02) | 0.19 (±0.02) |
| | Fall 14°C 10:14LD | 0.86 (±0.11) | 1.01 (±0.12) | 0.96 (±0.12) | 0.99 (±0.10) | 0.89 (±0.14) | 1.11 (±0.11) | 0.94 (±0.11) | 0.95 (±0.12) | 0.76 (±0.10) | 0.77 (±0.11) |
| | Winter 10°C 08:16LD | 0.96 (±0.08) | 1.13 (±0.08) | 0.99 (±0.12) | 1.14 (±0.10) | 0.98 (±0.11) | 0.93 (±0.10) | 1.01 (±0.10) | 1.00 (±0.10) | 0.96 (±0.10) | 1.18 (±0.10) |
| **Fat content FC** | Spring 20°C 16:08LD | 0.49 (±0.02) | 0.53 (±0.03) | 0.49 (±0.03) | 0.48 (±0.03) | 0.51 (±0.02) | 0.53 (±0.08) | 0.52 (±0.02) | 0.55 (±0.03) | 0.59 (±0.03) | 0.62 (±0.03) |
| | Fall 14°C 10:14LD | 0.54 (±0.02) | 0.56 (±0.02) | 0.54 (±0.01) | 0.56 (±0.02) | 0.55 (±0.03) | 0.57 (±0.02) | 0.56 (±0.02) | 0.58 (±0.03) | 0.61 (±0.02) | 0.64 (±0.02) |
| | Winter 10°C 08:16LD | 0.61 (±0.08) | 0.62 (±0.02) | 0.59 (±0.02) | 0.62 (±0.01) | 0.60 (±0.02) | 0.59 (±0.03) | 0.60 (±0.02) | 0.63 (±0.01) | 0.63 (±0.02) | 0.66 (±0.01) |

### *Egg load at emergence and volume of the eggs*

There was no significant effect of the population on the number of mature eggs at emergence (GLM: $\chi^2$=2.29; *df*=4, *P*=0.68) nor on the volume of eggs (GLM: $\chi^2$=5.16; *df*=2, *P*=0.17). In contrast, a significant effect of the condition was observed a larger number of eggs at emergence under winter conditions (GLM: $\chi^2$=9.53; *df*=2, *P*<0.01) as well a greater volume of the eggs (GLM: $\chi^2$=82.31; *df*=2, *P*<0.001), when compared to the other two conditions. Additionally, there were significant interaction effects of population and condition for the number of eggs (GLM: $\chi^2$=23.61; *df*=8, *P*<0.001) and the volume of the eggs (GLM: $\chi^2$=15.64; *df*=8, *P*=0.05), suggesting that the effect on the population was dependent of the condition under which females developed (Figure 3.4 A and Figure 3.4 B). Finally, the number of mature eggs increased with the size of parasitoids (GLM: $\chi^2$=1,147.54; *df*=1, *P*<0.001) (Figure 3.4 C), with larger individuals producing larger eggs (GLM: $\chi^2$=685.75; *df*=1, *P*<0.001) (Figure 3.4 D).

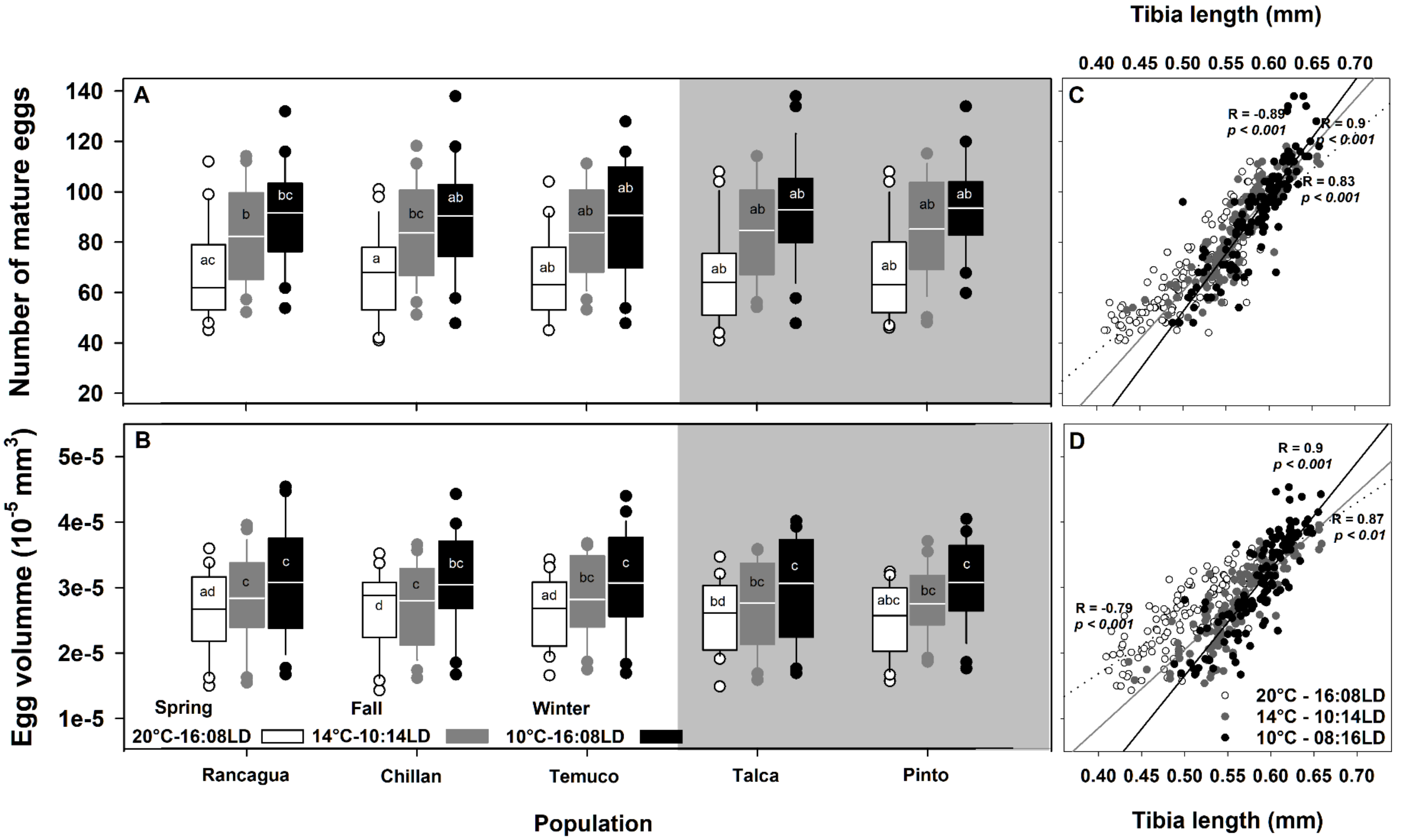

**Figure 3.4** Effect of the three conditions on the five populations of *A. platensis* for the following parameters: (A) Mean number of mature eggs, (B) Correlation between the number of eggs and the tibia length, (C) Mean volume of eggs in mm3 and (D) Correlation between the volume of the eggs and the tibia length. Twenty-five females at their emergence were used to measure these life-history traits. Box plots showed the median; lower and upper quartiles; whiskers: smallest and largest non-outlier observations; dots: outliers. Different letters indicate a significant difference between populations, conditions and sexes. Tukey HSD post hoc test ($P < 0.05$). The white part shows the mild winter localities and the grey part shows the cold winter localities.

## Discussion

The results indicate that among the five localities, the coldest were Pinto and Talca, which did not follow a north-south gradient. No diapausing individuals were found, regardless of the population or condition, with similar emergence rates, as proposed by our first and second hypotheses. In concordance with our third, fourth and fifth hypotheses, developmental times of *A. platensis* were slightly different among populations and however this had no effect on the survival of their progeny. Although we showed few differences in the physiology and life-history traits among populations, individuals from Pinto were larger, resisted lower temperatures, had slightly lower WC and higher FC values than individuals from the other populations. This was not at the expense of a lower fecundity in individuals from Pinto, as there were no differences in the number and volume of the eggs compared to other populations. Regarding the effect of the simulated conditions, we observed that an increase in temperature and photoperiod induced a shorter developmental time, a reduced size and body mass, smaller WC, FC, number and volume of eggs, as expected from hypothesis three, five and six, respectively. Finally, life-history traits responded differently in both sexes as in general, females were larger, heavier, more resistant to low temperatures and had higher WC than males.

Cereal crops in central-south Chile offer an interesting study model to evaluate the overwintering strategies, since the environmental conditions can change from favourable to inhospitable through the latitudinal gradient (Danks 2007). Unexpectedly, our results revealed no clear north-south latitudinal gradient in temperature. However, we may classify our localities as ‘cold winter’ and ‘mild winter’ on a transversal gradient, with the second southernmost locality of Pinto being the coldest. Diapause was not expressed in *A. platensis* under fall and winter conditions, even for populations from the coldest areas. This phenomenon can be explained as the fall and winter conditions we tested do not reach the threshold necessary to trigger diapause incidence (Tougeron et al. 2020). Low diapausing levels were observed in other parasitoids exposed to fall-like and winter-like laboratory conditions (Benelli et al. 2014, Tougeron et al. 2017). It has been recorded that some species such as *Aphidius rhopalosiphi* De Stefani-Perez and *A. matricariae* Haliday, show a decrease in winter diapause expression, due to warmer winters in Western France which could have modified diapause induction thresholds (Tougeron et al. 2017). Moreover, the parasitoids *Aphidius avenae* Haliday and *Aphidius ervi* Haliday, which are known to enter diapause in winter in the past, now have been reported to remain active in cereal fields (Andrade et al. 2016, Tougeron et al. 2017) suggesting that 'remaining active' as an overwintering strategy may become common in insects (Tougeron et al. 2017). In regions with mild winters, the absence of lethal frosts allows aphids and their parasitoids to remain active (Andrade et al. 2016, Tougeron et al. 2019). Consequently, the overwintering strategy of *A. platensis* probably also depends on the occurrence of its main host *R. padi*, the most abundant aphid species in winter (Alfaro-Tapia et al., *in preparation*). However, it is not clear whether the *A. platensis* populations lost their capability to express diapause, or if they do not possess the genetic and molecular constitution needed to diapause. Loss of diapause expression may be genetically determined in parasitoids when maintained in non-diapausing conditions within a few generations (Tougeron 2019, Tougeron et al. 2020). Rapid evolutionary changes in life-history strategies can occur under some environmental conditions through the process of selection or plastic adjustments Van Dyck et al. 2015, Tougeron et al. 2019) and our results can be attributed either to phenotypic plasticity or to local adaptation due to genetic changes. This suggests some new perspectives on the environmental thresholds needed to induce diapause in *Aphidius* species.

Physiological and life-history traits of the individuals from the five populations, showed that some traits differed between populations and within each population according to the temperature-photoperiod conditions. The processes of local adaptation and acclimation are often interdependent and different strategies allow species to occur over broad and variable environmental conditions (Blanquart et al. 2013, Merilä & Hendry 2014). In the present study we were able to observe consistent variation among populations related to the native temperatures where parasitoids were originally sampled. *A. platensis* from Pinto had a slower developmental time than Chillan population under winter conditions suggesting that slow development could be an adaptative response to cold environments, similar to what was observed in *Drosophila melanogaster* through clines or small regions (Austin & Moehring 2019) favouring an increase in performance with a decrease in temperature. Additionally, individuals from Pinto population were the biggest. Geographically, this pattern was observed in other taxa with increasing altitude and/or latitude (colder environments) (Chown & Klok 2003, Quicke 2012) which integrates physiological compromises in response to local conditions (Arnett & Gotelli 1999, Chown & Klok 2003), observing a significant advantage for survival at low temperature (Renault et al. 2003). Cold adaptation may vary among populations as a consequence of direct or indirect effects of selection. *A. platensis* from Pinto were more resistant to cold temperatures (i.e., lower $CT_{Min1}$ and $CT_{Min2}$) than the other populations. This difference coincided with the temperature conditions (e.g., total hours below 0°C) of their natal origin at the pre-cordillera area with higher altitude. Therefore, the differences observed among populations could be due to local conditions or genetic differentiation, perhaps due to limited gene flow favouring local adaptation (Blanquart et al. 2013). The Climate Variability Hypothesis (CVH) that describes how thermal limits vary across latitudes may explain our observations (Kellermann & van Heerwaarden 2019). Cold winter temperatures can help to predict local adaptations in *A. platensis*, suggesting that the association between minimum thermal tolerance and the minimum temperature of Pinto plays an important role in its distribution as a species, improving its fitness in its local environment (Kawecki & Ebert 2004). The slight decrease observed in WC in the population from Pinto could result from cold adaptation (Renault et al. 2003, Colinet et al. 2006), and may be associated with an increase in FC. It is known that the WC of insects resistant to cold is usually lower (Precht et al. 1973), with high FC and variations between populations (Chown & Nicolson 2004). Since insects use fat to fuel their overwinter energy demands (Sinclair & Marshall 2018), a higher FC is usually associated with higher cold tolerance (Sinclair 2015, Sinclair & Marshall 2018). Nevertheless, the responses of the other traits were similar in all populations (e.g., fecundity). Therefore, we provide evidence that the native environmental conditions may exert an important effect on intraspecific variation and together with natural selection, produce differences in some traits, which are finally translated into local adaptations (Blanquart et al. 2013).

Temperature and photoperiod are effective sources of plastic responses (Van Dyck et al. 2015). The survival of the individuals of the five populations does not seem to be affected by the exposure to low temperatures and short photoperiods during the larval development, contrary to what was found by Sigsgaard (2000) who observed the highest mortality at 8°C and 12°C in *A. ervi*, *A. rhopalosiphi*, and *Praon volucre* Haliday. As predicted, development at different temperature-photoperiod conditions induced a plastic response in the size/mass, increasing when temperature and photoperiod decreased. These results suggest a close relationship between temperature, size and developmental time, a pattern commonly referred to as the temperature-size rule (TSR) (Atkinson 1994), also observed for other *Aphidius* species (Colinet et al. 2007a b, Le Lann et al. 2011b). Strong correlations between body size/mass of parasitoids that emerged from different conditions and different measured traits were observed. The difference in thermal tolerance ($CT_{Min1}$ and $CT_{Min2}$) between conditions could result from developmental time differences. Individuals emerged from winter conditions better tolerated low temperatures showing an acclimation effect. Acclimation may require changes in WC and FC, in fact, a significant increase in WC was observed in *A. platensis* developed at low temperatures. This was also observed for the closely related species *A. colemani* (Colinet et al. 2006), as well as in other species (Renault et al. 2002). Cold tolerance is based on water content that remains unfrozen in many cold-hardened insects, allowing basal metabolism to continue at a low level (Block 2003, Colinet et al. 2006). Therefore, acclimation to low temperatures is characterized by an increase in water content (Lee 1991), compensated for by the loss of mass caused by fat use (Nedved & Windsor 1994). Insects primarily consume lipids during winter (Sinclair 2015) and commonly adjust their fat reserve and composition to thermal conditions to stabilize detrimental effects (Sinclair & Marshall 2018). Nevertheless, contrary to other studies, where the consumption of lipids reserves during cold exposure translates into a fitness cost (Colinet et al. 2006), the fat content in *A. platensis* does not seem to provide costs on life-history traits. Consequently, available fat reserve content is a key factor in the physiology of resource allocation for cold tolerance (Ellers & van Alphen 1997, Colinet et al. 2007c). Higher fat contents are advantageous for survival at low temperatures (Renault et al. 2003, Colinet et al. 2006), which could explain the prevalence of *A. platensis* in winter throughout Chile.

In addition, it has been proposed that the fitness of a female parasitoid is determined by the number of viable offspring produced (Roitberg et al. 2001), which is strongly dependent on body size (Ellers & Jervis 2003, Jervis et al. 2003) and fat content. The fecundity in *A.*

*platensis* was highly sensitive to the conditions of temperature and photoperiod; developmental plasticity exists in eggs in response to the condition tested. In winter conditions, *A. platensis* produced more and larger eggs. These traits were highly correlated with FBM and followed a similar response to fat content. Large adult body size is often associated with increased resource carried over from the larval stage, and this is manifested as higher levels of energy reserves and high fecundity (Ellers & van Alphen 1997, Ellers et al. 1998, Ellers & Jervis 2003). Other Aphidiinae parasitoids such as *A. rhopalosiphi* showed a similar pattern as *A. platensis,* with more eggs produced at the emergence and larger eggs at low temperatures (10°C) (Le Lann et al. 2011b).

It is known that females and males undergo changes on different life-history traits when facing low temperatures (Zamani et al. 2007, Le Lann et al. 2011a b). Independently of *A. platensis* population and condition differences in cold tolerance between sexes were observed. Females were more resistant to cold than males, contrary to what was reported for *A. avenae* and *A. rhopalosiphi* (Le Lann et al., 2011a) but similar to what was reported for *Aphidius gifuensis* (Ashmead) by Liu et al. (2016). In Hymenoptera, males are haploid and females are diploid, suggesting that haplodiploidy plays an important role in thermal tolerance differences between sexes as diploid cells can repair the damage through DNA recombination (Roux et al. 2010). Another explanation is that females generally live longer than males, however, we did not find differences in *A. platensis* (*unpublished data*), therefore, this is not a general rule, making it difficult to draw a conclusion about sex (Colinet & Boivin 2011). Females have higher WC than males in all populations, however the FC was similar. Also, these results may be related to the size/mass of the individuals, confirming that larger and fatter adults are more tolerant to colder environments when compared to smaller individuals (Colinet et al. 2007a b) which could explain why females were more tolerant. However, this may not be a general rule since some smaller individuals have been shown to be more resistant to cold in the winter than in spring for some *Aphidius* species (Ismail et al. 2012, Tougeron et al. 2016).

Understanding the consequences of climate variability on parasitoids is fundamental for biological control programmes (Hance et al. 2007). Mild winter temperatures could be favourable for increased parasitoid populations that depend on the availability of a suitable host over the winter period (Santos et al. 2019). It is known that an early appearance of parasitoids in the field could facilitate parasitism in winter and early spring, vital for effective biological control (Zepeda-Paulo et al. 2013, Tougeron et al. 2017, 2018b, Santos et al. 2019). The maintenance of the population of *A. platensis* in the field is dependent on their acclimation capacity and reproduction over winter, and the absence of associated fitness cost suggests that *A. platensis* can remain active during winter acting against aphid pests in cereal fields in Chile. In particular, *R. padi* could be used as an alternative host for *A. platensis,* allowing the build up of a large population of parasitoids during the winter and could be useful to decrease populations of *S. avenae,* the most important aphid for cereals in spring at the beginning of the season (Zepeda-Paulo et al. 2013, Ortiz-Martínez & Lavandero 2018). Therefore, the influence of seasonal weather and particularly temperature-photoperiod conditions on parasitoids should be considered to improve our understanding of their effectiveness as biological control agents in the field.

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## *Conflict of interest*

None to declare.

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**Appendix 3.1. Table S3.1.** General results of the different models tested for populations of *A. platensis* along the latitudinal gradient of Chile, when faced to three different conditions of temperature and photoperiod. For each level, the number of freedom degrees (df), the statistic test and the p value are represented.

| **Subsection/Results** | **Variable** | **Statistic test** | **Fixed factor** | ***df*** | **Test Statistic**[a] | ***P value*** |
|---|---|---|---|---|---|---|
| ***3.2 Emergence rate, Diapause expression, and developmental time*** | Emergence rate | GLM Binomial | Population | 4 | $\chi^2=0.69$ | $P=0.95$ |
| | | | Condition | 2 | $\chi^2=0.42$ | $P=0.81$ |
| | | | Population × Condition | 8 | $\chi^2=0.32$ | $P=1.00$ |
| | Non-emerged mummies | GLM Binomial | Population | 4 | $\chi^2=0.01$ | $P=1.00$ |
| | | | Condition | 2 | $\chi^2<0.01$ | $P=1.00$ |
| | | | Population × Condition | 8 | $\chi^2=0.04$ | $P=1.00$ |
| | Total developmental time | LR Survival analysis | Population | 4 | $\chi^2=0.01$ | $\boldsymbol{P=3.06\ e^{-07}}$ |
| | | | Condition | 2 | $\chi^2<0.01$ | $\boldsymbol{P<2.2e^{-16}}$ |
| | | | Population × Condition | 8 | $\chi^2=0.04$ | $\boldsymbol{P=1.72\ e^{-05}}$ |
| ***3.3 Fresh body mass (FBM) and Size*** | Fresh Body Mass | GLM Gaussian | Population | 4 | $\chi^2=1.02$ | $P=0.91$ |
| | | | Condition | 2 | $\chi^2=1705.46$ | $\boldsymbol{P<2.2e^{-16}}$ |
| | | | Sex | 1 | $\chi^2=41.89$ | $\boldsymbol{P<9.66^{-11}}$ |
| | | | Population × Condition | 8 | $\chi^2=21.10$ | $\boldsymbol{P=0.01}$ |
| | | | Population × Sex | 4 | $\chi^2=0.72$ | $P=0.95$ |
| | | | Condition × Sex | 2 | $\chi^2=4.58$ | $P=0.10$ |
| | | | Population × Condition × Sex | 8 | $\chi^2=4.53$ | $P=0.81$ |
| | Tibia length | GLM Gaussian | Population | 4 | $\chi^2=16.45$ | $\boldsymbol{P=0.00}$ |
| | | | Condition | 2 | $\chi^2=689.22$ | $\boldsymbol{P<2.2e^{-16}}$ |
| | | | Sex | 1 | $\chi^2=70.56$ | $\boldsymbol{P<2.2e^{-16}}$ |
| | | | Population × Condition | 8 | $\chi^2=4.57$ | $P=0.80$ |
| | | | Population × Sex | 4 | $\chi^2=2.64$ | $P=0.62$ |
| | | | Condition × Sex | 2 | $\chi^2=2.05$ | $P=0.36$ |
| | | | Population × Condition × Sex | 8 | $\chi^2=3.66$ | $P=0.89$ |
| ***3.4 Critical thermal minima*** | $CT_{Min1}$ | GLM Gaussian | Population | 4 | $\chi^2=9.46$ | $\boldsymbol{P=0.05}$ |
| | | | Condition | 2 | $\chi^2=77.05$ | $\boldsymbol{P<2.2e^{-16}}$ |
| | | | Sex | 1 | $\chi^2=11.86$ | $\boldsymbol{P=0.00}$ |
| | | | Population × Condition | 8 | $\chi^2=2.64$ | $P=0.95$ |
| | | | Population × Sex | 4 | $\chi^2=0.65$ | $P=0.96$ |
| | | | Condition × Sex | 2 | $\chi^2=0.01$ | $P=1.00$ |
| | | | Population × Condition × Sex | 8 | $\chi^2=1.25$ | $P=1.00$ |
| | | | FBM[b] | 1 | $\chi^2=6.42$ | $\boldsymbol{P=0.01}$ |
| | $CT_{Min2}$ | GLM | Population | 4 | $\chi^2=308.43$ | $\boldsymbol{P<2.2e^{-16}}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | Gaussian | Condition | 2 | $\chi^2$=1618.56 | **$P<2.2e^{-16}$** |
| | | | Sex | 1 | $\chi^2$=176.78 | **$P<2.2e^{-16}$** |
| | | | Population $^{x}$ Condition | 8 | $\chi^2$=27.88 | **$P=0.00$** |
| | | | Population $^{x}$ Sex | 4 | $\chi^2$=10.79 | **$P=0.03$** |
| | | | Condition $^{x}$ Sex | 2 | $\chi^2$=1.91 | $P$=0.38 |
| | | | Population $^{x}$ Condition $^{x}$ Sex | 8 | $\chi^2$=13.37 | $P$=0.10 |
| | | | FBM[b] | 1 | $\chi^2$=727.93 | **$P<2.2e^{-16}$** |
| ***3.5 Water and Fat content*** | Water content | GLM Gaussian | Population | 4 | $\chi^2$=22.11 | **$P<0.00$** |
| | | | Condition | 2 | $\chi^2$=110.04 | **$P<2.2e^{-16}$** |
| | | | Sex | 1 | $\chi^2$=7.22 | **$P<0.01$** |
| | | | Population $^{x}$ Condition | 8 | $\chi^2$=17.41 | **$P<0.03$** |
| | | | Population $^{x}$ Sex | 4 | $\chi^2$=2.47 | $P$=0.65 |
| | | | Condition $^{x}$ Sex | 2 | $\chi^2$=1.91 | $P$=0.39 |
| | | | Population $^{x}$ Condition $^{x}$ Sex | 8 | $\chi^2$=7.53 | $P$=0.48 |
| | | | Tibia length[b] | 1 | $\chi^2$=282.69 | **$P<2.2e^{-16}$** |
| | Fat content | GLM Gaussian | Population | 4 | $\chi^2$=32.17 | **$P=1.76e^{-06}$** |
| | | | Condition | 2 | $\chi^2$=11.94 | **$P=0.00$** |
| | | | Sex | 1 | $\chi^2$=0.70 | $P$=0.40 |
| | | | Population $^{x}$ Condition | 8 | $\chi^2$=8.74 | $P$=0.36 |
| | | | Population $^{x}$ Sex | 4 | $\chi^2$=0.25 | $P$=0.99 |
| | | | Condition $^{x}$ Sex | 2 | $\chi^2$=0.28 | $P$=0.87 |
| | | | Population $^{x}$ Condition $^{x}$ Sex | 8 | $\chi^2$=1.16 | $P$=1.00 |
| | | | Tibia length[b] | 1 | $\chi^2$=30.69 | **$P=3.03e^{-08}$** |
| ***3.6 Egg load at emergence and volume of the eggs*** | Egg load | GLM Binomial Negative | Population | 4 | $\chi^2$=2.29 | $P$=0.68 |
| | | | Condition | 2 | $\chi^2$=9.53 | **$P=0.01$** |
| | | | Population $^{x}$ Condition | 8 | $\chi^2$=23.61 | **$P=0.00$** |
| | | | Tibia length[b] | 1 | $\chi^2$=1147.54 | **$P<2.2e^{-16}$** |
| | Volume of the eggs | GLM Gamma | Population | 4 | $\chi^2$=5.16 | $P$=0.27 |
| | | | Condition | 2 | $\chi^2$=82.31 | **$P<2.0e^{-16}$** |
| | | | Population $^{x}$ Condition | 8 | $\chi^2$=15.64 | **$P=0.05$** |
| | | | Tibia length[b] | 1 | $\chi^2$=685.75 | **$P<2.0e^{-16}$** |

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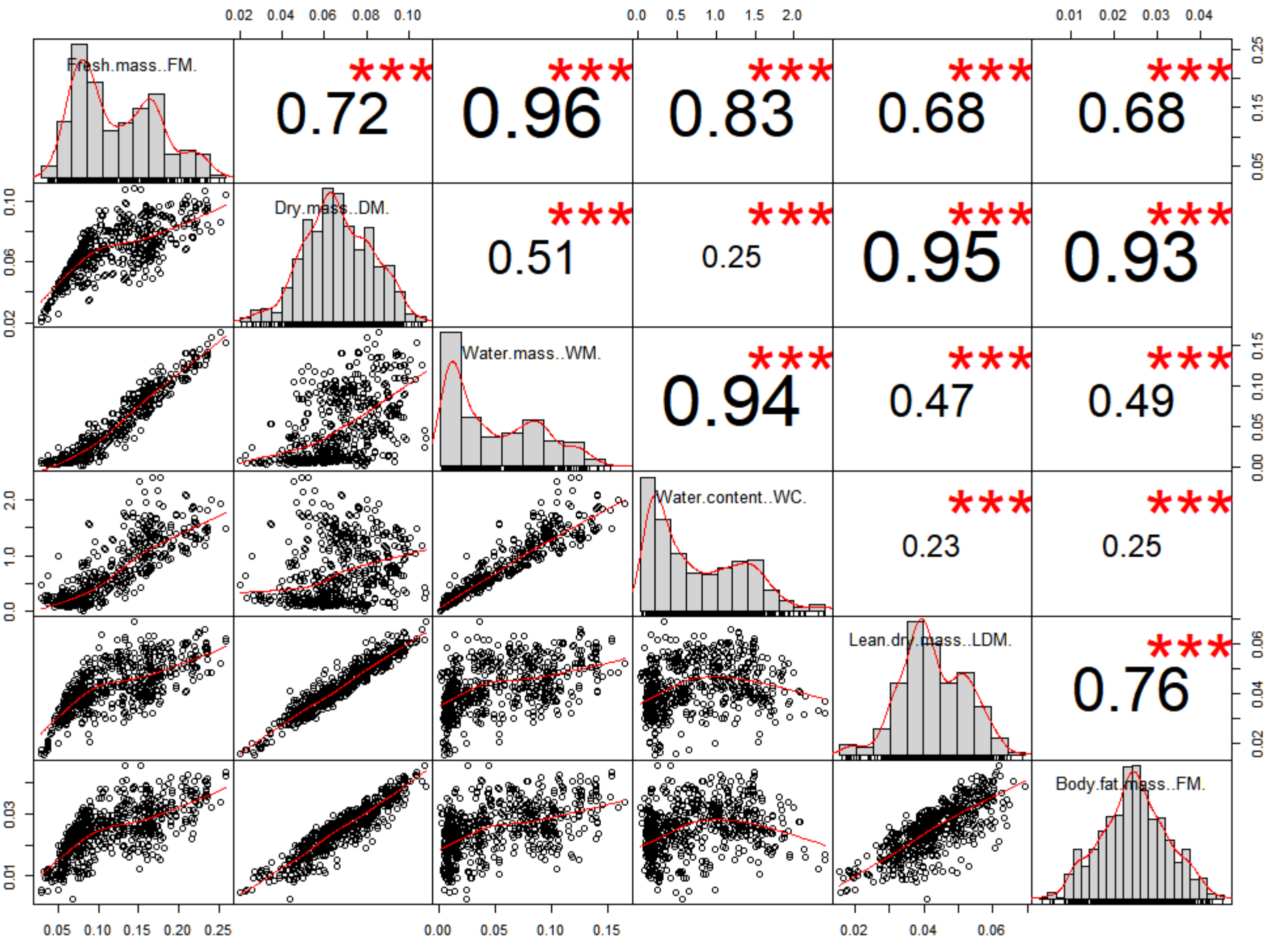
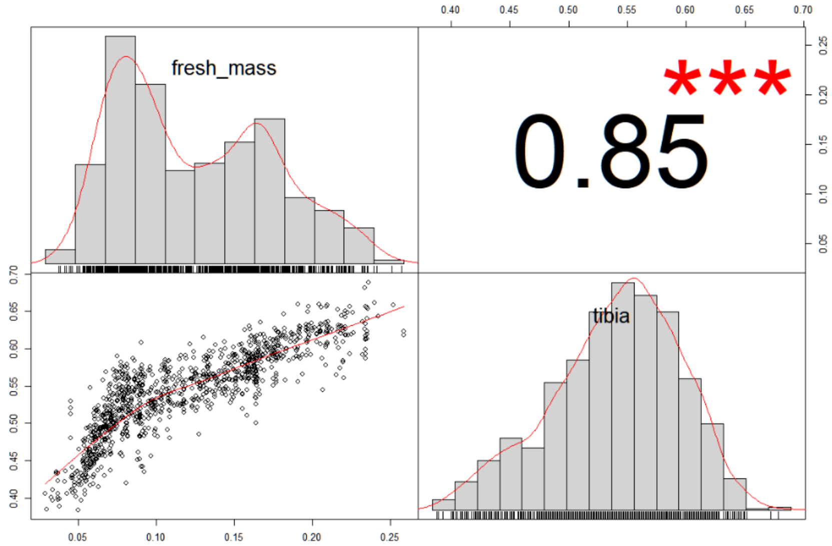
[a] Wald tests $\chi^2$ values given for GLM and logistic-rank test (LR) for survival analysis followed by Tukey's HSD multiple comparison $P < 0.05$

[b] Variable used as covariate in the analysis

**Appendix 3.2. Figure. S3.2.** Global Spearman correlations between changes in physiological parameters (***$p < 0.05$) of parasitoids (males and females). Coefficients of correlations (R) and probabilities of R Pearson tests are shown.



**Appendix 3.3. Figure S3.3** Global Spearman correlations between fresh body mass and tibia length (***p < 0.05) of parasitoids (males and females). Coefficients of correlations (R) and probabilities of R Pearson tests are shown.



# Effect of host species and intraspecific competition on winter and summer diapause in *Aphidius ervi*

**Armando Alfaro-Tapia, Cécile Le Lann, Joan van Baaren, and Blas Lavandero**

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## Abstract

Ectotherms, like insects, generally resist to harsh conditions inducing resource scarcity by diapause, which can happen in both winter and summer allowing them to synchronize their life cycle with the availability of resources. Photoperiod and temperature where shown to be the main cues inducing winter diapause whereas summer diapause was generally driven by weak resource density . However, winter and summer diapause were rarely studied on the same species. Therefore, identifying the cues acting in both winter and summer diapause in a same species is importantto develop a comprehensive view of insect resistance according to the harshness of the environement and therefore to predict the consequences of climate changes on populations. In this study, winter and summer diapause incidence of five Chilean populations of the parasitoid *Aphidius ervi,* sampled in cereal fields in different environmental conditions, was evaluated. For winter diapause, two hosts, = *Acyrthosiphon pisum* and *Sitobion avenae* were used. For both winter and summer-like conditions, female parasitoids were exposed to low (10 aphids) and high (75 aphids) host densities of *A. pisum* and to the absence or presence of intra-specific competition (1 and 10 competing females) at high host density. No difference was found in the proportion of diapausing offspring in all of the 5 populations (5%) for winter diapause. A significant host effect was shown, with higher diapause rates/levels in *A. pisum* (20%) than in *S. avenae* (5%). The diapause incidence increased with decreasing host density at winter-like conditions whereas it increased with the level of competition experienced by female parasitoids at summer-like conditions. In conclusion, diapause may be adaptive and other mechanisms such as phenotypic plasticity and bet-hedging in diapause allow parasitoids to synchronize their life cycle with seasonal climatic changes and host resources.

**Key words:** Climatic gradient, host effect on diapause, scarcity of resource; maternal competition

# Introduction

Understanding the mechanisms by which ectotherms respond to environmental heterogeneity is a fundamental goal of evolutionary ecologists (Denlinger 2003; Hance et al. 2007). Ectotherms are mostly vulnerable to environmental and seasonal variability (Angilletta Jr 2009). Insects as small ectotherms are known to live in a wide range of thermal climates and have to adopt different strategies to resist unfavorable conditions (Lehmann et al., 2014; Stange & Ayres, 2010; Thomas et al., 1992). In temperate areas, seasonality occurs between fluctuations of photoperiod and temperature that limit the resource availability and are reliable cues providing induction of stress response (Tauber et al. 1986; Angilletta Jr 2009; Klapwijk et al. 2010; Colinet et al. 2015). Insects experience some degree of spatial and temporal environmental variation (Danks 1991; Potter et al. 2013; Williams et al. 2015) and have evolved specific responses (Gienapp et al. 2008; Kong et al. 2019). Such variation includes short and large climatic scale, according to the latitude or altitude (Archibald et al. 2010; Hut et al. 2013). In the high latitudes (cold latitudes), seasonal variation in photoperiod is a more consistent factor than temperature, On the contrary, in low latitudes (warm latitudes) where seasonal variation in the day length is small-scale, temperature changes may have a higher predictive value than the photoperiod (Hut et al. 2013). Therefore, insects living in the temperate zones have the capability to regulate different physiological functions such as metabolism, development, and reproduction to synchronize their life cycles with seasonality in space and time (Bale and Hayward 2010; Stange and Ayres 2010; Williams et al. 2015).

To cope with the different environmental conditions, insects use a wide variety of behavioral and physiological strategies such as migration, remaining active and reproduce or entering diapause (Masaki 1980; Tauber et al. 1986; Denlinger 2003; Tougeron 2019; Saunders 2020; Tougeron et al. 2020a). Diapause is a dynamic state of decelerated or arrested morphological development accompanied by a major shutdown in metabolic activity, genetically determined with the neuro-hormonal system as a mediator and its expression is subject to both environmental and genetic factors (Tauber et al. 1986; Denlinger 2003; Saunders 2020). In temperate regions, diapause is often used to avoid winter conditions, however it is also used to avoid hot, dry summers and periods of food shortage (Denlinger

2009) The main environmental cues inducing winter diapause are the decrease of day length and temperature (Tauber et al. 1986; Leather et al. 1995; Tougeron 2019; Saunders 2020). On contrary, summer diapause is mostly induced by a decrease in food resource availability, overcrowding, or high competition for a limited resource on a patch in warm environmental conditions (Masaki 1980; Tougeron et al. 2018b). However, it has also been observed that temperature and photoperiod affect the maintenance and termination of summer diapause (Masaki 1980). Moreover, cues related to induction, maintenance, and termination such as host scarcity and maternal effects on summer diapause are not well understood yet (Tougeron et al. 2018b).

Diapause is an adaptation that parasitoids have evolved to synchronize their life cycle with their hosts and it is a resistance stage many parasitoids use to overwinter and over summer (Tougeron et al. 2018b). Commonly in parasitoids species diapause is facultative, and only part of the population enters in winter and/or summer diapause (Polgar and Hardie 2000; Tougeron et al. 2018b). Aphid parasitoid species diapause in the third final larval instar (prepupa diapause) (Polgár et al. 1991; Tougeron et al. 2017b), but the sensitive cues differ between embryonic, first (*Aphidius nigripes*) and the second larval instar (*A. ervi*) (Brodeur and McNeil 1989; Christiansen-Weniger and Hardie 1999). For parasitoids living in temperate latitudes, winter diapause occurs at short photoperiods and low temperatures (Brodeur and McNeil 1989; Christiansen-Weniger and Hardie 1999; Tougeron et al. 2017b, 2018c; Saeed et al. 2020), however, its rate of incidence varies among populations from harsh winter than mild winter climates (Tougeron et al. 2018c). This was observed in *A. ervi* where the diapause incidence was higher in Canadian populations (≈ 90%) than in French populations (< 20%) (Tougeron et al. 2018c, 2019b), proving the importance of photo-thermal regimen in the response of *A. ervi* to express diapause. In addition, for a given parasitoid species winter diapause is induced earlier and at a lower environmental threshold in high (cold) latitudes (Winterhalter and Mousseau 2007; Paolucci et al. 2013). Contrary to winter diapause, summer diapause is triggered mainly by a decrease in host and food resources, as well as female competition, play a more important role (Saulich and Musolin 2018; Tougeron et al. 2018b) that can be mediated by increase of photoperiod and temperature (Masaki 1980).

Winter and summer diapause expression may be sensitive to any modification in seasonal host densities and food shortage (Saunders 1966; Polgár et al. 1991; Polgar and Hardie 2000; Tougeron et al. 2020a). In *Aphidius* parasitoids, where the aphid host continues its develop during parasitism, the aphid represents the entire physiological environment (Polgár et al. 1991). Changes in host quality (i.e., species, size, morph) can modify both winter and summer diapause expression (Polgar and Hardie 2000). For instance, it has been observed that *Aphidius matricariae* did not enter diapause parasitizing the anholocyclic aphid species *Aulacorthum solani* Kalt., *Aphis gossypii* Glover and *Myzus ascalonicus* Doncaster (Hemiptera, Aphididae) compared with parasitoids developed on *Myzus persicae* Sulzer (Hemiptera, Aphididae) (Polgár et al. 1995; Polgar and Hardie 2000), probably in response to aphid hormonal content (e.g., juvenile hormones and Ecdysteroids). For instance, diapause was induced when parasitized aphids by *A. ervi* where topically applied with the ecdysteroid agonist RH-5849 hormone (Christiansen-Weniger nd Hardie 1999). In addition, fat body of the host play an important role in energy storage and utilization demand during diapause of parasitoids (Hahn and Denlinger 2010) and large hosts could have better resources. Moreover, maternal perception of abiotic and biotic conditions could contribute to summer diapause induction (Tougeron et al. 2020a). through intra or interspecific competition in *Aphidius avenae* and A*phidius rhopalosiphi* (Tougeron et al. 2018b). However, these cues inducing both winter and summer diapause are rarely investigated within of one species, so that a comparison of the response of the same species to different cues inducing winter and summer diapause may provide new insights to understand the mechanism of diapause development.

The aphid parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) is a generalist species which can be found in many crops around the world (Van Emden and Harrington 2017). It was introduced into Chile from Europe in the 70's as a biological agent to control cereal aphids (Zuñiga et al. 1986) such as the grain aphid *Sitobion avenae* (Fabricius) (Hemiptera, Aphididae), however it is also successful parasitizing the pea aphid *Acyrtosiphum pisum* (Harris) (Hemiptera, Aphididae) on legumes (Zepeda-Paulo et al. 2013), its main host (Schellhorn et al. 2002). In Chile, *A. pisum* hosts on legumes could serve as reservoirs for *A. ervi* in autum/winter (Daza-Bustamante et al. 2002), since *Rhopalosiphum padi* (Linnaeus) (Hemiptera, Aphididae), the predominant aphid species attacking cereal crops in winter is not a suitable host for *A. ervi* (Zepeda-Paulo et al. 2013), and *S. avenae,* a suitable host species for *A. ervi* is present in winter at low abundance (Chapter II) increasing its population at the beginning of the spring (Ortiz-Martínez and Lavandero 2017). *A. ervi* is known to enter in diapause the winter in response to short day lengths and low temperatures in different regions of the world with harsh climate (Christiansen-Weniger and Hardie 1999; Polgar and Hardie 2000; Tougeron et al. 2017b, 2018c, b). However, in areas with mild winters such as Chile with available resources (anholocyclic cereal aphids which remain active during the winter) (Chapter II), this species was shown to express a decreasing diapause rate following winter warming (Tougeron et al. 2017b).

In this study, we first evaluated whether diapause incidence in Chilean natural populations of *A. ervi* vary along a latitudinal gradient with relatively mild winter temperatures. Second, we assessed whether similar biotic factors could trigger/induce winter and summer diapause expression of this species under winter and summer photoperiod-thermal regimes, respectively. Therefore, we tested the following hypotheses: (1) the frequency of diapause should be higher in populations from colder localities than from mild ones. (2) The incidence of winter diapause in parasitoid offspring will be higher on *A. pisum* than on *S. avenae*. Besides, the level of diapause could be linked to the scarcity of the resources, which can also be experienced when host density decreases or when competition among parasitoids increases, thus: (3) When females face low aphid densities, the diapause incidence may increase in winter and summer conditions, and 4) The incidence of diapause may be higher when females experience an increase in competition levels in winter and summer conditions.

## Material and methods

### Biological material

#### *Aphids*

The grain aphid *Sitobion avenae* and the pea aphid *Acyrthosiphon pisum* were collected from cereal fields (wheat and oat) and legumes (alfalfa) respectively from the Region del Maule, Talca (S 35°24′, W 71°40′) at the end of winter season of 2018. The two aphid species were maintained under controlled laboratory conditions at 20°C, 16 :8 light: dark (L:D) of photoperiod and 60 ± 5% R.H on oat *Avena sativa* and beans *Vicia faba,* respectively and refreshed in April of 2019. This allowed us the continued reproduction of aphids, parasitoids, and their use in the experimental bioassays.

### *Parasitoids*

First, aphid mummies (i.e., aphid exoskeletons containing the parasitoid pupae) were collected of the five populations following a north–south latitudinal gradient in the central-south valley of Chile d during winter field samplings between July and August of 2018 on cereal crops: one field in Rancagua (34°09′S, 70°44′W and 469m), seven fields in Talca (35°22'S, 71°50'W and 189m), two fields in Chillan (36°32′S, 72°04′W and 154m), two in Pinto (36°41'S, 71°46'W and 519m), and four fields in Temuco (38°55'S, 72°02'W and 114m). These mummies were individually isolated in Eppendorf tubes of 1.5 mL with a small hole in the lid to allow ventilation until parasitoid emergence and maintained in laboratory conditions at 20 ± 1°C, 16 :8 L:D and 65 ± 5 % RH. Emerging parasitoids were identified to the species level using taxonomic keys (Starý 1995). Parasitoids identified as *A. ervi* were used to establish a laboratory population on *S. avenae* on oat pots in Plexiglas cages of 0.40m$^3$ and fed with honey solution (30%) and water. The laboratory populations were started with about 20 to 40 individuals for each population. These populations were used to evaluate diapause induction in a climatic gradient. In addition, since we found a low level of diapause for the 5 populations, new mummies of *A. ervi* were collected in Talca from aphids on alfalfa and cereal fields in autumn of 2019. Mummies were placed in 1.5 ml Eppendorf tubes and taken back to the laboratory until the emergence. After the emergence, parasitoids identified as *A. ervi* originating from both types of fields were mixed and reared on *A. pisum* on beans at the same conditions mentioned above and refreshed in spring of 2019 and autumn of 2020. It was shown that *A. ervi* showed a similar high fitness on both host species (Zepeda-Paulo et al.

2013). This laboratory population was used to evaluate the host effect, host density and female competition level on offspring diapause of *A. ervi*.

## Winter diapause incidence in a climatic gradient: effect of photoperiod and temperature

We quantified diapause incidence for each population previously described in two constant conditions of photoperiod and temperature; 8:16 LD at 10°C (winter condition), and 16:8 LD at 20°C (non-inducing diapause condition).Winter-like condition was chosen as it induces diapause in different *Aphidius* species (Polgár et al. 1991; Tougeron et al. 2017b, 2019b) and the other condition is known for not inducing winter diapause in populations from mild winter climates (Langer and Hance 2000; Polgár and Hardie 2000; Tougeron et al. 2017a). For the experiments, individual mummies of each population of *A. ervi* were isolated from plants in Eppendorf tubes and checked for emergence twice daily. Adult parasitoids were sexed and mated. To ensure mating, four newly emerged females were placed with three males of the same age in a Petri dish of 4.5cm Ø for 24h. During this time, they were supplied with a honey solution diluted in water (30%) and then, stored in groups of four females in a Petri dish of 4.5cm Ø supplied with water and diluted honey solution. After this time, under non-diapausing condition, the four females were exposed together for 24 h to 100 ± 10 *S. avenae* of second instar on a wheat pot in a Plexiglas cage. Potentially parasitized aphids coming from each population, were distributed randomly by groups of ≈25 aphids on four wheat pots among two different conditions (two pots per condition) in climate chambers (Sanyo, Osaka, Japan). Pots consisted in plastic tubes (L = 16cm, Ø = 5cm) filled with vermiculite substrate, closed with 100% nylon organdy mesh. All females used in the experiments were one to two days old and naïve in terms of oviposition experience. Females had never been in contact with plants or aphids before the experiments and were used only once. Three days prior to a test, apterous parthenogenetic aphids of second and third instars of both species were synchronized on wheat (Brodeur and McNeil, 1989) to be used in the experiments. All potentially parasitized mummies were held under the experimental conditions until the mummies were dissected to determine the incidence of diapause 15 days after last emergence. To do that, we carefully made a tiny on hole on the mummy without damaging the individual inside under a binocular microscope (Nikon SMZ800N, 480x). The dead immatures/adults or diapausing golden-yellow plump pre-pupa were recorded (Tougeron et al. 2017b). In addition, the emergence rate was estimated as the mean proportion of parasitoids which emerged from the mummies formed (Henry et al. 2010) and the mortality rate was estimated as the mummies with non-emerged adults.

## Host species effect on winter diapause incidence

To evaluate the host species effect (*A. pisum* and *S. avenae*) on the incidence of diapause, mummies were isolated in 1.5mL Eppendorf tubes until parasitoid emergence. In the same way and in the same conditions as in the previous experiment, four *A. ervi* mated females were allowed to parasitize together 100 ± 10 synchronized aphids of the same age and size within the same cohort of each aphid species. Aphids were exposed to parasitoids while feeding on their host plant in a Plexiglas cage: fava bean and wheat for *A. pisum* and *S. avenae*, respectively. After the oviposition period (24 h), potentially parasitized aphids were sorted randomly by groups of 25 on four pots of beans and wheat plants respectively and covered with a transparent ventilated plastic cylinder (L = 30 cm, Ø = 10 cm). Each pot was considered as one replicate and two pots of each aphid host were incubated under the two experimental photoperiod/temperature conditions described above in climate chambers (Bioref-38, Pitec S.A, Chile). After three days, incubated aphids were checked daily for mummification. When plants started to wilt, aphids were transferred to another plant with a small paintbrush. Formed mummies were individually confined into Eppendorf tubes and intact aphids which had not become mummies within three days after the last mummification were excluded from the observation. The experiment was replicated 10 times until 200 mummies were obtained for each host and condition. The presence of diapause was evaluated as previously described.

## Effect of host density on winter and summer diapause incidence

To test the effects of host density on diapause incidence, *A. ervi* coming from *A. pisum* were exposed to two levels of host density: 10 (low) and 75 aphids (high). These densities correspond to the number of aphids on wheat shoots in the fields (Roschewitz et al. 2005) and were chosen to have a situation with low and high host density, representing summer and winter conditions. *A. ervi* females are known to be able to adjust their oviposition strategies in response to increased host density through increasing parasitism and decreasing superparasitism (Bai and Mackauer 1992). Fifty aphids per female per day is the highest critical density (realized fecundities for 24h) at which the parasitoid can maximize its parasitism success (He and Wang 2008). Naive mated females were individually introduced into experimental arenas to parasitize aphids. This experiment was replicated 20 times at low density and 15 times at high density using different females for each repetition. This experiment was done at both winter (10°C and 16:8 LD) and summer inducing diapausing conditions (24°C and 8:16 LD) of temperature and photoperiod. Mummy formation as well as parasitoid emergence was checked daily. Diapause and mortality rates from non-emerged mummies were evaluated as previously described.

## Females' competition on winter and summer diapause incidence

Single mated females were placed in a Petri dish (L = 1.3 cm, Ø = 5.5 cm) with diluted honey solution for feeding alone (without competition) or with 9 other females (with competition) under winter or summer like climatic conditions for 48 h, following (Tougeron et al. 2018b). This parasitoid density (10 females) induces summer diapause in other aphid parasitoids species: *A. rhopalosiphi* and *A. avenae* (Tougeron et al. 2018). Females from the competition treatment were randomly chosen and were offered individually 75 aphids in an experimental arena (L = 30 cm, Ø = 10 cm). This experiment was repeated with winter (10°C and 16:8 LD) and summer (24°C and 8:16 LD) conditions of photoperiod and temperature using fifteen different females for each competition treatment having a total of 60 females.

## Statistical analyses

All statistical analyses were carried out with R software (R Development Core Team, 2019). Generalized linear mixed-effects models (GLMMs) with binomial distributions and a logit-link function for proportional data were fit to establish differences in all experiments. First, to evaluate the winter diapause incidence in a latitudinal gradient, the effect of the population origin (five levels), and the tested conditions (two levels) were used as fixed factors. The effect of host species *A. pisum* and *S. avenae* (two levels) and the tested condition (winter and non-diapausing condition) were used as fixed factors to assess the effect of host species on winter diapause incidence. Besides, to evaluate if the same biotic cues such host density and female competition are involved on winter and summer diapause induction, the effect of the host density (low and high density) and two tested conditions (winter and summer conditions) were used as fixed factors. Moreover, differences in the diapause offspring according to female competition (two levels) and two tested conditions (winter and summer conditions) was evaluated. In all experiments, in addition to the proportion of diapause incidence (diapausing or non-diapausing), the emergence, and mortality rate (dead larvae/adults) were also set as response variables. In all models, the replicate was included as random factor. GLMMs analyses were applied because it can easily account for complex data structures with various levels and effectively deal with unbalanced data sets (van de Pol and Wright 2009, Fox and Weisberg 2011). The best model was chosen using the Akaike criterion and performing an ANOVA in the car package (Fox et al. 2009). Pairwise comparisons were developed using ‘Tukey’ tests, correcting for multiple comparisons by the ‘single-step’ method using Multcomp package (Hothorn et al. 2008).

# Results

## Winter diapause incidence in a climatic gradient: effect of photoperiod and temperature

Winter diapause was induced in *A. ervi* coming from cereal aphids exposed to the 10°C and 8:16 LD temperature-photoperiod condition but only at a low rate. The proportion of parasitoids entering winter diapause was not significantly affected by population origin ($\chi^2$ = 0.05; *df* = 4, *P* = 0.10), however, all tested populations expressed diapause at a low rate (around 5%) under winter conditions ($\chi^2$ = 49.53; *df* = 1, *P* = 0.001) without interactions between populations and conditions (Figure 4.1). The proportion of emerging individuals was similar among populations ($\chi^2$ = 0.12; *df* = 4, *P* = 0.10) but was higher in the non-diapause inducing condition (0.92 ± 0.01) than in the winter diapause-inducing condition (0.84 ± 0.01) ($\chi^2$ = 17.19; *df* = 1, $P < 0.001$) without interactions between population and condition factors. Similarly, mortality rate was similar in all populations ($\chi^2$ = 0.11; *df* = 4, *P* = 0.10) but this proportion increased in winter conditions 0.10 ± 0.01 compared to non-diapause inducing conditions 0.08 ± 0.01 ($\chi^2$ = 17.19; *df* = 1, $P < 0.001$) without interaction between factors.

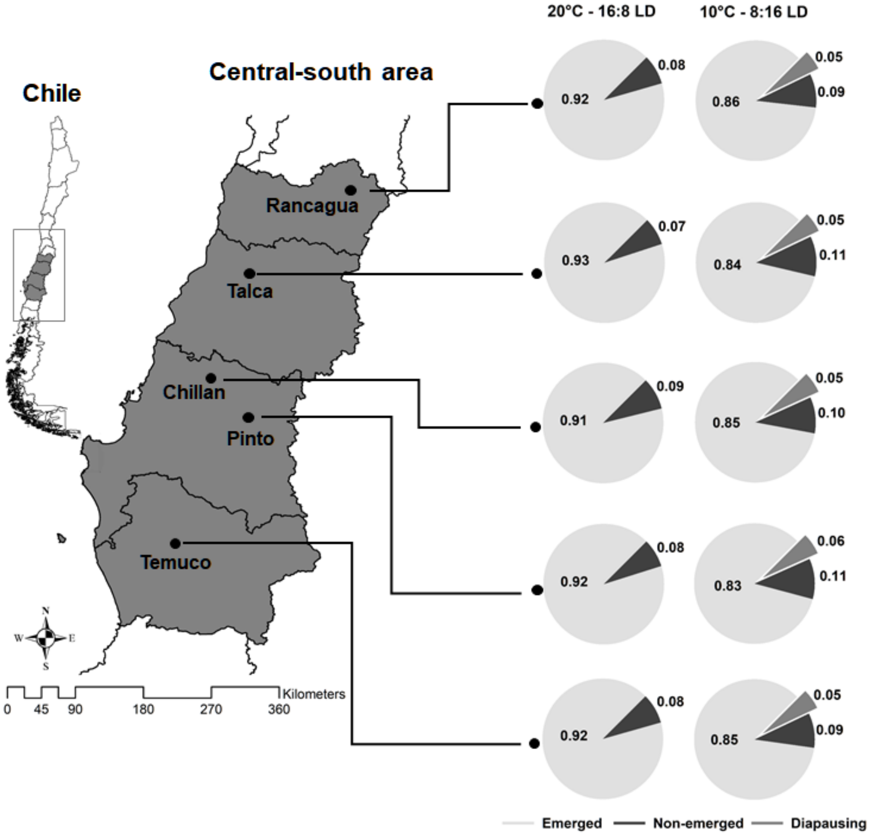

**Figure 4.1.** Map of sampling locations in central-south of Chile and proportions of emerged adults, dissected non emerged mummies containing a dead larvae/adult and dissected non emerged mummies containing a prepupal diapausing individual produced by *Aphidius ervi* females sampled from cereal fields on *Sitobion avenae* and tested at two different conditions of temperature and photoperiod: winter (10°C and 8:16 LD) and non-diapausing conditions (20°C and 16:8 LD). A total of 1282 mummies were formed in this laboratory experiment for both conditions tested.

## Host species effect on winter diapause incidence

The incidence of diapause was significantly higher on *A. pisum* than on *S. avenae* ($\chi^2$ = 31.38; *df* = 1, $P < 0.001$), in winter conditions ($\chi^2$ = 102.20; *df* = 1, $P < 0.001$), without interactions between host species effect and condition effect (Figure 4.2). Proportion of parasitoid emergence was significantly lower on *A. pisum* (0.83 ± 0.03) than on *S. avenae* hosts (0.89 ± 0.03) ($\chi^2$ = 8.99; *df* = 1, $P < 0.01$). Parasitoid emergence rate in winter conditions were lower (0.79 ± 0.02) than in the controlled conditions (0.94 ± 0.01) ($\chi^2 = 40.23$; $df = 1$, $P < 0.001$). There was a significant interaction between aphid species and tested condition ($\chi^2 = 4.42$; $df = 1$, $P = 0.04$), with a lower proportion of emerging individuals on *A. pisum* at winter conditions (0.73 ± 0.01). There were no differences in parasitoid mortality according to the aphid species tested ($\chi^2 = 0.65$; $df = 1$, $P = 0.42$) with an average of 0.07 ± 0.01 on *A. pisum* and 0.08 ± 0.01 on *S. avenae* nor tested condition ($\chi^2 = 1.00$; df = 1, $P = 0.32$) with an average of 0.06 ± 0.01 in the controlled condition and 0.08 ± 0.01 in winter condition without interactions between factors.

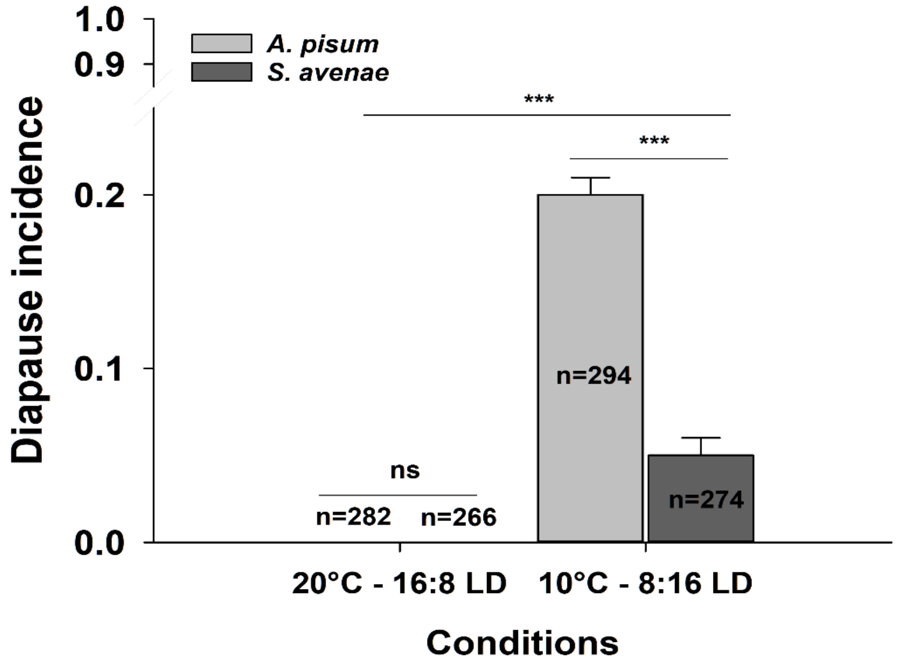

**Figure 4.2.** Proportion of diapause incidence in *A. ervi* parasitizing *A. pisum* and *S. avenae* at two conditions of temperature and photoperiod (winter and laboratory-controlled conditions). The number of mummies is provided on the figure for each aphid host species and condition. A total of 1116 mummies were formed. Significant differences are shown (Post hoc Tukey test; $P < 0.05$): < 0.001 '***', < 0.01 '**', < 0.05 '*', and ns 'no significant'.

## Effect of host density on diapause incidence

Proportion of diapause incidence was higher at winter conditions than at summer conditions ($\chi^2 = 39.58$; df = 1, $P < 0.001$). In the same way, there was a higher proportion of diapause incidence at low than at high host aphid density ($\chi^2 = 8.06$; df = 1, $P < 0.01$), reaching maximums of 0.20 ± 0.03 when females were exposed to low host density compared to the 0.11 ± .0.01 in winter and 0.02 ± 0.01 at high host density in summer condition (Figure 4.3). The mean proportion of parasitoids emerged from summer conditions was higher than in winter conditions ($\chi^2 = 50.54$; *df* = 1, *P* < 0.001). At the same time, there was lower emergence rate at low density than at high density ($\chi^2 = 22.34$; *df* = 1, *P* < 0.001) without interaction between condition and density treatment factors. A higher mortality rate occurred at winter than at summer conditions ($\chi^2 = 8.07$; *df* = 1, *P* < 0.01) as well as at low host density than at high host density ($\chi^2 = 11.78$; *df* = 1, *P* < 0.001) without interactions between factors (Table 4.1).

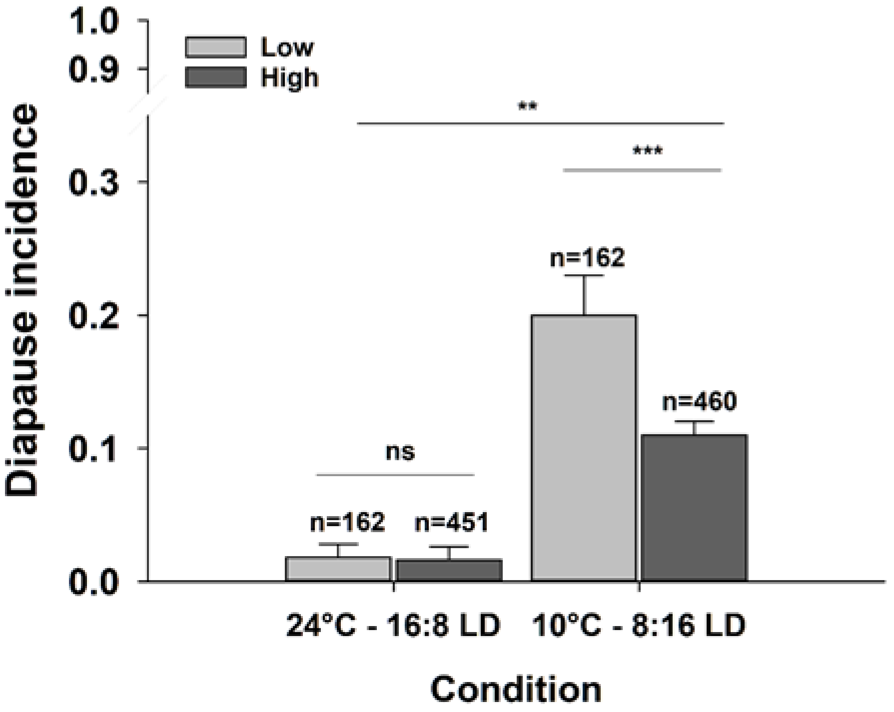

**Figure 4.3.** Proportion of diapause incidence in *A. ervi* parasitizing *A. pisum* at different conditions (winter and summer) and two levels of host density. The number of mummies formed is provided on the figure for each aphid host and condition. A total of 1235 of mummies were formed. Significant differences are shown (Post hoc Tukey test; *P* < 0.05): < 0.001 '***', < 0.01 '**', < 0.05 '*', and ns 'no significant'.

## Incidence of Females' competition on diapause

The diapause incidence was higher at summer inducing diapause condition ($\chi^2$ = 14.24; df = 1, $P < 0.001$): at 0.11 ± 0.01 than at the winter condition 0.05 ± 0.02. Similarly, diapause incidence was lower 0.06 ± 0.01 without female competition than at 0.10 ± 0.01 when there is competition ($\chi^2$ = 6.51; df = 1, $P = 0.01$), with an interaction between factors ($\chi^2$ = 9.82; df = 1, $P < 0.01$), meaning that diapause incidence was lower in summer condition when females were unexposed to competition than in the other combinations (Fig. 4). The proportion of parasitoids emergence increased at summer condition with an average of 0.87 ± 0.01 compared than winter condition 0.73 ± 0.02 ($\chi^2$ = 57.35; *df* = 1, $P < 0.001$). In contrary, the level of female competition did not affect the proportion of emergence ($\chi^2$ = 57.35; *df* = 1, $P$ = 0.08) without interaction between factors. The proportion of mortality was lower 0.15 ± 0.01 at winter condition than at 0.70 ± 0.01 at summer condition respectively ($\chi^2$ = 31.57; *df* = 1, $P < 0.001$), however there was not a significantly effect according to exposed and unexposed females' competition ($\chi^2$ = 0.21; *df* = 1, $P = 0.65$) without interaction between factors (Table 4.1).

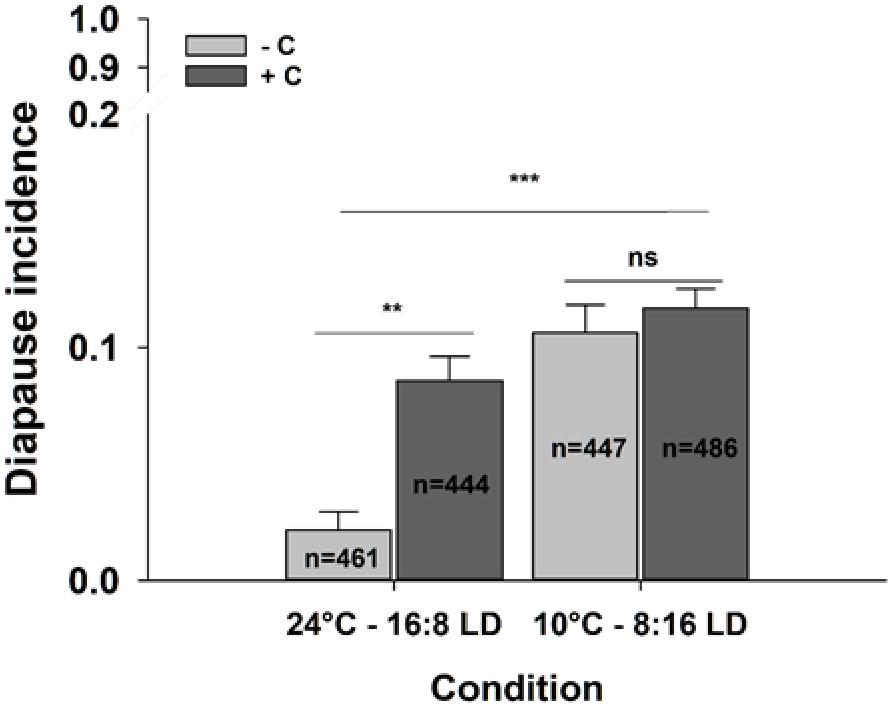

**Figure 4. 4.** Proportion of diapause incidence in *Aphidius ervi* parasitizing *A. pisum* at two conditions (winter and summer) in relation to female parasitoid competition (- C=without competition and + C=with competition). The number of mummies formed is provided on the figure for each aphid host and condition. A total of 1838 of mummies were formed. Significant differences are shown (Post hoc Tukey test; $P < 0.05$): < 0.001 '***', < 0.01 '**', < 0.05 '*', and ns 'no significant'

**Table 4. 1.** Total number of mummies formed and emerging pattern of *A. ervi* parasitizing *A. pisum* at different levels of host density and competition (- C = without competition and + C = with competition) between parasitoid females at 10°C and 8:16 LD and 24°C and 16:8 LD temperature and photoperiod regimen. Values in brackets are proportions. Different letters indicate a significant difference between treatments (Tukey HSD post-hoc test (P < 0.05).

| Parameter | Condition | Treatment | Total No. mummies | Mummies Emerged [1] | Dead Parasitoid [2] |
|---|---|---|---|---|---|
| **Host density** | **Winter** | **Low** | 162 | 95 (0.58 ± 0.03) a | 35 (0.22 ± 0.03) a |
| | | **High** | 460 | 353 (0.76 ± 0.02) b | 59 (0.13 ± 0.01) b |
| | **Summer** | **Low** | 162 | 136 (0.84 ± 0.02) c | 23 (0.14 ± 0.02) b |
| | | **High** | 451 | 406 (0.90 ± 0.01) c | 37 (0.09 ± 0.03) b |
| **Female competition** | **Winter** | **- C** | 447 | 333 (0.74 ± 0.01) a | 68 (0.15 ± 0.01) a |
| | | **+ C** | 486 | 350 (0.72 ± 0.02) a | 79 (0.16 ± 0.02) a |
| | **Summer** | **- C** | 444 | 398 (0.90 ± 0.01) b | 37 (0.08 ± 0.01) b |
| | | **+ C** | 461 | 395 (0.85 ± 0.01) b | 27 (0.06 ± 0.01) b |

[1] The remaining values correspond to number of emerged individuals
[2] The remaining values correspond to number of alive plus diapausing individuals

# Discussion

This study aimed to understand the main abiotic and biotic factors affecting the overwintering strategies of the parasitoid *A. ervi* in Chile. According to what was expected, *A. ervi* populations did not show variation in their diapause incidence according to their geographic locations. Moreover, we observed very low rates of diapause (about 5%) on cereal aphids. Our data present the evidence that diapause in *A. ervi* may be mediated by the aphid host at low temperatures and short photoperiod conditions as diapause rate were four times higher in *A. pisum* (20%) than in *S. avenae* (5%). Furthermore, our results show that low host density (resource scarcity) induce higher levels of diapause of *A. ervi* development on *A. pisum* at winter condition confirming in part our third hypothesis. Contrary as expected, we did not observe an effect of increasing maternal competition on winter diapause induction. However, an effect of female competition was observed on summer diapause incidence. In this sense, our study provides evidence that in addition to environmental cues, diapause induction in parasitoids may be differentially influenced by the aphid species, host density, and female competition in winter and summer conditions.

## Latitudinal gradient and proportion of winter diapause

Insects confronted to cold environments usually need to adjust their life cycle to match seasonal changes in their environment by entering in diapause (Polgar and Hardie 2000; Bale et al. 2002; Denlinger 2003, 2009; Bale and Hayward 2010; Tougeron et al. 2020a). To do that, the majority of insects rely on temperature and photoperiodic cues for diapause (Visser et al. 2010; Kong et al. 2019; Saunders 2020), therefore diapause incidence varies between populations along environmental gradients (Danks 1991; Paolucci et al. 2013; Barletta and Dantas 2016; Zhang et al. 2017). Contrary to what was expected, we observed very low levels of diapause in *A. ervi* for all the tested populations under winter conditions, which represents standard conditions that typically induce diapause at higher rate in other populations of this parasitoid wasps in other climatic areas (Christiansen-Weniger and Hardie 1999; Langer and

Hance 2000; Tougeron et al. 2018c, 2019b) including mild winter ones (Tougeron et al. 2017b, 2018c). A latitudinal trend is generally observed for other parasitoids species such as *Nasonia vitripennis* Ashmead (Hymenoptera: Pteromalidae) and *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae), where the diapause incidence reached high levels and occurs sooner at north latitudes compared to south populations which experience mild winters (Paolucci et al. 2013; Zhang et al. 2017). This area is characterized by the absence of harsh winter and in the last years, it has been suffering relative temperature homogenization as a consequence of climate change with an increase of warming in the Andes of +0.25°C/decade (Falvey and Garreaud 2009). These environmental characteristics suggest a weak response of *A. ervi* from these populations to photoperiod and temperature (Tougeron et al. 2018c), thus, environmental thresholds for inducing high diapause rates are not reached in Chile. Similar responses were observed in French populations of *A. ervi* (Tougeron et al. 2017b, 2018c), suggesting that abiotic conditions are not the main cues inducing winter diapause compared to biotic signals (see below). In Chile this parasitoid species was introduced in the 1970's from France as part of a classical biological control (Zuñiga et al. 1986) and *A. ervi* seems to be a genetically homogeneous population distributed in space and time among different aphid hosts (Zepeda-Paulo et al. 2015). In addition, anholocyclic aphid species have been available throughout the winter on cereal crops which allows aphid parasitoids to remain active (Chapter II) as well as in late spring and early summer in Chile (Zepeda-Paulo et al. 2013; Ortiz-Martínez and Lavandero 2017). A similar pattern was observed in mild climate areas in absence of lethal frosts (Tougeron et al. 2018a), leading to a modification in overwintering strategies in some parasitoid species (Andrade et al. 2016; Tougeron et al. 2017b).

## Effect of the host species on diapause induction

Our results show that in addition to environmental conditions, diapause induction in *A. ervi* was influenced by the host species attacked. Interestingly, even when *A. ervi* parasitize different aphid species, it exhibits greater plasticity for diapause induction, being higher on *A. pisum* than on *S. avenae*, suggesting that parasitoids do not respond the same way to both abiotic pressure (photoperiod and temperature) and host species. A similar pattern was observed in other areas with mild winter conditions where *A. ervi* expressed higher levels of diapause on *A. pisum* (around 30%) (Tougeron et al. 2019a) than *S. avenae* (around 10%) (Tougeron et al. 2017b). Thus, it seems that diapause expression of this parasitoid species on different host species can affect some life-history traits through winter. Any change in host quality across seasonal changes may be a selective pressure that may modify diapause expression in parasitoids (Polgár et al. 1991; Polgár et al. 1995; Polgar and Hardie 2000). One explanation for our findings is that the pea aphid *A. pisum* is larger than grain aphid *S. avenae* which would give to parasitoid more reserves during the diapause. It has been shown that variation in aphid size is correlated with food quality (Cohen et al. 2005) and small aphids have fewer available resources for the parasitoid development based in different life-history traits. In parasitoids, the stock of reserves is provided by the and reserves accumulated prior to diapause are crucial for providing metabolic substrates during diapause and for post-diapause processes (Hahn and Denlinger 2010) even if the metabolism rate decreases (Denlinger 2003).

The non-production of sexual morphs in *S. avenae* may also explain our results. It has been demonstrated that obligated parthenogenetic reproduction in *S. avenae* is predominant since their introduction in Chile because of the mild climate (Figueroa et al. 2005, 2018), while in *A. pisum* it may still exist. In Chile, it has been recorded that parthenogenetic and sexual reproductive mode occur in cold areas (Martel et al. 2020). Therefore, physiological changes in *A. pisum* may exist when they are exposed to low temperatures that could trigger diapause at a higher rate. For instance, parasitoids are also able to respond to aphid hosts in different ways as a consequence of their endocrine activity as a signal to enter in diapause (Polgár et al. 1995; Polgar and Hardie 2000). On the other hand, legumes probably do not harbor sufficient density of *A. pisum* to maintain active parasitoid populations in winter, suggesting that diapause in *A. ervi* may be adaptive to its main host (Christiansen-Weniger and Hardie 1997). In contrary, cereals are abundant resources on which *S. avenae* could be at sufficient densities for maintaining a large part of *A. ervi* population active in winter (Alfaro-Tapia *in prep*). Therefore, *A. ervi* could use this cue as stimulius to express winter diapause present in perennial legumes during the period when cereal crops are not available (Daza-Bustamante et al. 2002).

## Effect of host density on diapause incidence

Our results showed a negative correlation between host density and diapause induction, but not for summer diapause. Diapause induction in *A. ervi* seems to arise from the perception of host scarcity, providing the first evidence of host density inducing winter diapause in aphid parasitoids. Since our induction to diapause in this experiment was carried out in winter and summer conditions, it is possible that female parasitoids had captured the cues of resources scarcity directly in winter conditions. On contrary, in summer conditions the scarcity of resources may be captured by sensing competition (see below). Based on our results it is possible to suggest that the production of diapausing inducing by host scarcity in winter may be associated with an abrupt shortage of hosts in winter. The subsequent abundance of other host species has not been tested though (e.g., *S. avenae*). In Chile it has been observed that other aphid cereal species can maintain active populations of *A. ervi* during winter (Chapter II). Thus, entering in diapause at the beginning of winter in an alternative host such as *A. pisum* on legumes may allow parasitoids to synchronize their life cycle with seasonal climatic changes and host resources, since it has been shown that entering diapause earlier in the winter would prevent the overexploitation of an abundant resource in the future (Polgár et al. 1995). However, legumes probably do not harbor sufficient density of *A. pisum* to maintain active parasitoid populations in winter, suggesting that diapause in *A. ervi* may be adaptive to its main host (Christiansen-Weniger and Hardie 1997). Therefore, *A. ervi* could use this cue as stimuli to express winter diapause present in perennial legumes during the period when cereal crops are not available (Daza-Bustamante et al. 2002). On the other hand, we found a small proportion of diapausing individuals in summer without an effect of host density. This result is in accordance to what was found by Tougeron et al (2018c) to other aphid parasitoid species, where *A. rhopalosiphi* and *A. avenae* did not express summer diapause according to the number of hosts offered, suggesting that in the genus *Aphidius*, summer diapause is induced by the perception of female competition (see below) instead as host scarcity.

## Effect of maternal competition on diapause incidence

In *A ervi,* we observed that diapause induction is influenced by the perception of direct intraspecific competition among female parasitoids in summer conditions, but this signal could not select the winter diapause induction. Our results suggest that parasitoids may respond differently to the risk of intraspecific competition under winter and in summer seasonal conditions. These findings can be explained due to the fact that winter parasitoid population is small and this signal is probably never encountered in nature, since alternative hosts may exist in cereal crops during winter time (Chapter II). While in summer, a decrease in populations of cereal aphids occur at the beginning of this season in the central south of Chile (Ortiz-Martínez and Lavandero 2017). Another explanation is that the main goal of parasitoids is to survive harsh winter conditions whereas in summer, it is to avoid host shortage. On the other hand, a different pattern was found in the summer diapause induction where *A. ervi* seems to detect environmental risks according to maternal experience. Similar results were observed in *A. rhopalosiphi* and *A. avenae*, where an increase in temperatures was associated with an increase in competition between females inducing higher diapausing levels (Tougeron et al. 2018b), suggesting that parasitoids may respond differently to the environmental risk. These variation in the response in summer and winter condition may be explained by the way of producing diapause offspring by female parasitoids. It has been known that mothers in some species of *Aphidius* that lay diapausing offspring only do this in part of their progeny, other produce only diapause progeny and others mothers may produce a mix of both (Tougeron et al. 2018b, 2020b). Thus, this response can be induced by the female parasitoids to enhance offspring fitness since the proportion of each offspring may be adjusted to the probability of encountering a stochastic environment (Menu et al. 2000; Marshall and Uller 2007) resulting in bet-hedging strategies. A bet-hedging trait is expected to evolve when the environment which parasitoids face will take in the immediate future is unpredictable (Hairston and Fox 2013), allowing to ensure its synchrony with its host (He et al. 2010). This strategy has been observed in other arthropod species such as *Rhagoletis cerasi* (Diptera: Tephritidae) (Moraiti and Papadopoulos 2017) and the mite *Halotydeus destructor* Tucker (Arachnida, Penthaleidae) (Cheng et al. 2019) as a response to low quality of food and by environmental conditions experienced by parents respectively. In addition, it has been shown that maternal effects on winter diapause in *Aphidius* species may be significant but remains marginal compared to other environmental stimuli such as photoperiod and temperature (Langer and Hance 2000).

## Conclusion

The variability of stimuli that induce diapause in *A. ervi* may guarantee optimal exploitation of the available resources and offspring numbers surviving environmental harsh conditions. This result is consistent with the existence of several mixed signals inducing diapause in *A. ervi*, where dissociated mechanisms are involved in winter and summer diapause, but with weak levels in this species in Chile. These results can be due to low genetic variability observed between individuals of *A. ervi* from different aphid hosts and geographic zones (Zepeda-Paulo et al. 2015). Hence, it is likely that these parasitoid wasps may use different strategies to parasitize and exploit different host permitting the parasitoid to adequately assess seasonal changes in the environment.

### *Author's contributions*

AAT, JVB, CLL and BL conceived the ideas and designed methodology; AAT analyzed the data; AAT wrote the first draft. All authors contributed critically to the drafts and gave final approval for publication.

### *Disclosure*

The authors declare no conflict of interest.

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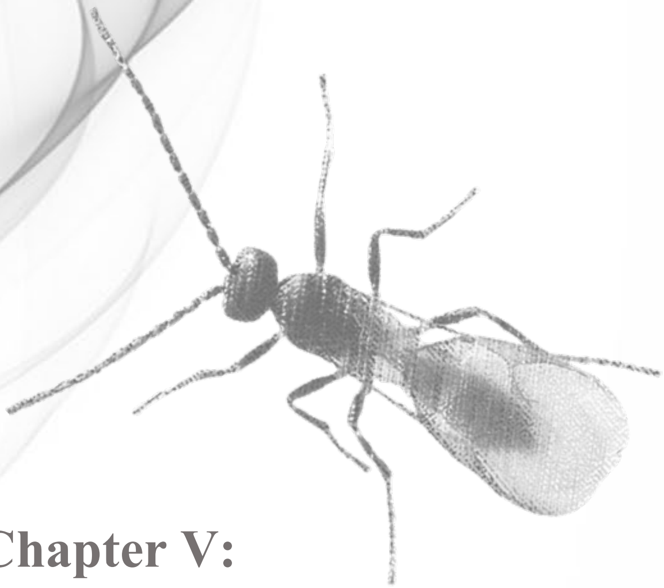
# Part 4
# General discussion



## Chapter V:

- General discussion
- Research perspectives
- Conclusion

# General discussion

## Overview

Understanding how climate influences insects' population regulation through species interactions is an important question in ecology. Host-parasitoid systems are among the most important ecological interactions affected by environmental changes due to the high trophic position of parasitoids, leading to the potential for the difference in phenological responses (seasonal strategies) and range modifications (Godfray 1994; Bale and Hayward 2010; Kehoe et al. 2018; Tougeron et al. 2020a). Diapause is an adaptation that allows parasitoids to synchronize with seasonal and host-resources changes (Polgár and Hardie 2000). It was shown that it is altered in some species in a changing environment (Brodeur and McNeil 1989; Tougeron et al. 2017, 2018, 2020). Changes in diapause expression can occur either through genetic adaptations or through phenotypic plasticity when facing new conditions, with consequences in the community structure, as well as the ecosystem functioning (Tougeron et al. 2020a; Le Lann et al. 2021). Throughout this Thesis, several determinants and ecological drivers have been explored in a guild of aphid parasitoids along a latitudinal gradient in the central south valley of Chile. We tested whether environmental conditions along this gradient led to a higher diapause incidence on high latitudes (colder) compared to low latitudes (warmer) and that both with a field sampling and with lab analyses. In addition, we explored whether diapause is regulated by biotic stimulus (i.e., host effect, host availability, and maternal intraspecific competition) in addition of abiotic stimuli (i.e., photoperiod and temperature), using laboratory and field approaches and their consequences on community stability.

Aphid parasitoids and their hosts are organisms with a wide distribution, living in different climatic conditions, many of which are found in cereal crops. Parasitoids are the main biological regulatory agents of aphid populations in different agroecosystems (Godfray 1994). Thus, variability in the parasitoid populations can affect aphid pest regulation (Neil and Jervis 2007). The extensive geographic distribution of this biotic interaction makes this an interesting model for the study of latitudinal patterns. (**Chapter 2**). Environmental variability may affect parasitoid species distributions, phenology, fecundity, and overwintering strategies with effects on community composition, and on the strength of biotic interactions. Usually, parasitoid species are exposed to stressful temperatures in their natural environments due to seasonal cycles. As other ectotherms, parasitoids have limited ability to regulate their body temperature and have thus required a range of strategies to support life in thermally stressful environments either through acclimation (plasticity) or genetic adaptation (**Chapters 3 and 4**). Here we present a framework for elucidating the abiotic and biotic effects on seasonal strategies of parasitoids that integrates a mechanistic description of life-history trait responses that characterize the phenology of many ectotherms.

Based on the intensity of winter, the sampled localities were categorized as either cold, mild, or warm winter areas, over a transversal gradient considering their inter-annual variability (**Chapters 2 and 3**). It is well known that differential responses to temperatures should allow species to cope with different environments (Le Lann et al. 2021). However, very little is known about the mechanisms by which temperature affects overwintering strategies that operate at the individual level, and how much translates into direct and indirect effects on their populations and communities. (**Chapters 3 and 4**). We predicted that diapause incidence varies along the climatic gradient with more diapause incidence in the coldest areas. Therefore, a decrease of parasitoid activity in the coldest areas would be observed during the winter period affecting the network structure composition. Contrary to what we expected, the species of parasitoids recorded here did not show a clear effect of the latitudinal gradient in diapause expression. Low levels of diapause incidence (around 4%) were observed along the latitudinal gradient in Chile. Diapause was observed only in three of the nine parasitoid species, *Aphidius ervi*, *A uzbekistanicus* and *Praon volucre* and interestingly one hyperparasitoid species *Phaenoglyphis villosa* (**Chapter 2**), and therefore host–parasitoids interactions in cereal crops along the gradient did not differ in terms of food-web composition. Differences observed for the three studied areas were only in terms of the relative abundances of species inside the guilds with spatial and temporal variations, mainly for the less abundant species.

Seasonal overwintering in insects represents an adaptation to stressful environments (Tauber et al. 1986) and in parasitoid species short photoperiods and low temperatures are the main cues inducing winter diapause (Brodeur and McNeil 1989; Langer and Hance 2000; Tougeron et al. 2017, 2019a). Employing *Aphidius platensis* and *Aphidius ervi* as study models, we assessed the role of photoperiod and temperature, the main drivers of diapause induction, on diapause incidence of five Chilean populations along the climatic gradient (**Chapters 2 and 3**). These species were recorded as the most abundant species in winter along the central-south valley of Chile corresponded to the area of cereal production (**Chapter 2**). Contrary to our hypothesis, none of the five populations of *A. platensis* expressed diapause at any tested condition (8:16 LD (Light: Dark, h) at 10°C, 10:14 LD at 14°C, and 16:8 LD at 20°C) representing winter, fall and summer conditions respectively (**Chapter 3**). Usually, winter and fall conditions trigger diapause induction in other aphid parasitoids from temperate areas (Brodeur and McNeil 1989; Langer and Hance 2000; Tougeron et al. 2017) suggesting that diapause induction thresholds are not reach for *A. platensis*.

A deeper understanding of the role of acclimation and local adaptation among parasitoid populations may complement information on the impact of climate on species distribution via the thermal biology of their individuals (e.g., thermal tolerance). Indeed, if the individuals do not enter in diapause, it means that they remain active during the while winter and need to be protected against cold spells. To increase knowledge in this research area, the present study examined patterns of thermal tolerance in five populations of *A. platensis* developing at different conditions of temperature and photoperiod. Specifically, we tested the hypotheses that the $CT_{Min}$, defined as the temperature of loss of coordinated muscle function is lower in populations originating from the coldest climates. Our results showed that both local adaptation and phenotypic plasticity (acclimation) may contribute to temperature tolerance, with possible role of several concomitant physiological adjustments such as water and fat content in mediating differences in thermotolerance along a latitudinal gradient (**Chapter 3**).

For the parasitoid species *A. ervi*, low levels of diapause were expressed. These results suggest that diapause induction thresholds are not reached by the environmental cues (photoperiod and temperature) in the central-south valley of Chile (**Chapters 3 and 4**) but that other cues such as aphid host species, host availability and maternal competition could also play a more dominant role (**Chapter 4**), essential for synchrony between parasitoids and their hosts. Diapause in parasitoids may be influenced by the host species (Brodeur and McNeil

1989). Our results provide evidence that *A. ervi* was influenced by the host species attacked, as it exhibits a greater diapause level when developing on *Acyrthosiphon pisum* than on *Sitobion avenae* suggesting plasticity for diapause induction (**Chapter 4**). Moreover, we compared the influence of host density and female competition on induction of winter and summer diapause. Diapause in *A. ervi* was influenced by host density, especially in winter, providing evidence that resource availability affects winter diapause in aphid parasitoids, a phenomenon previously observed for other parasitoid insects such as *N. vitripennis* (Saunders et al. 1970). Finally, the adaptive significance of maternal effects has been increasingly recognized in diapause induction (Mousseau and Dingle 1991). This work provides some insight of the perception of direct intraspecific competition among parasitoid females in summer conditions affecting diapause induction, however, this effect was not observed during winter conditions. In consequence, during summer, the female competition experience may be translated into variation in diapause expression of their offspring for the case of summer diapause (**Chapter 4**).

## Overwintering strategies in response to geographic variation

Insects of temperate regions are under strong selection to synchronize their life cycles to the seasonal changes in abiotic and biotic conditions. This selection is likely to vary spatially along latitudinal gradients, leading to different strategies to respond to their life-cycle regulation (Frenne et al. 2013), which in turn may destabilize trophic interactions and food web composition (Tougeron et al. 2020a). This study did not find either strong evidence of a clear climatic gradient, related to Central Chile's latitudinal variation nor response in diapause rates, as we found low levels of diapause incidence (around 4%), probably due to the fact that cold areas are not cold enough to induce diapause. Hence, available evidence does not provide support for the hypothesis that diapause in parasitoids increases with increasing latitude in mild winter areas such as Central Chile (**Chapters 2 and 3**). These results were confirmed by our laboratory experiments where diapause in *A. platensis* was not found for any of the populations tested and with low levels of diapause for the species *A. ervi* (around 5%) when faced with winter conditions inducing diapause in other aphid parasitoid populations (**Chapters 3 and 4**). Individuals within the aphid parasitoid guild were active during winter suggesting that the strategy of the major part of the parasitoid populations was to overwinter as active adults. The absence and/or low levels of diapause may be due to winter conditions in the central-south valley of Chile (**Discussed in Chapters 2, 3 and 4**). Recent studies suggest that the central-south valley of Chile has undergone relative inter-annual homogenization in temperatures as a consequence of climate change with a strong influence of the frontal system in central and southern Chile (Falvey and Garreaud 2009; Mutz et al. 2021). In the north-west of France, following winter warming, a decrease in diapause expression in some parasitoid populations in both natural and laboratory conditions was observed (Tougeron et al. 2017, 2018a, 2020a). In contrast to our results, some studies using other species of parasitoids such as *Nasonia vitripennis* Ashmead (Hymenoptera: Pteromalidae) and *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) as models, showed high levels of diapause incidence at north latitudes characterized by severe climatic condition compared to south populations which experienced mild winters (Paolucci et al. 2013; Zhang et al. 2017). Therefore, the current low diapause incidence in parasitoids could be understood as a response to mild winters happening in the central-south valley of Chile, which also allow the continuous host availability throughout the winter season, since the aphids *Rhopalosiphum padi*, *Sitobion avenae* and *Metopolophium dirhodum* were present in cereal crops throughout all the period. Previous studies have also recorded active anholocyclic cereal aphids under mild winters throughout the seasons in West Europe (Langer et al. 1997; Dedryver et al. 2001; Andrade et al. 2015), allowing resources for some parasitoids species to overwintering as active adults.

In winter, physiology and life-history traits vary along geographic gradients allowing individuals that remain active to cope with different environments, where thermal tolerance plays an important role when insects face unfavorable conditions (Turnock and Fields 2005; Angilletta Jr 2009). Variation in photoperiod and temperature along geographic gradients is expected to produce a plastic response and local adaptation of natural populations depending on their thermal tolerances (Begon et al. 2006; Blanquart et al. 2013; Merilä and Hendry 2014). We predicted that individuals from cold latitudes developing in different abiotic conditions (winter, fall and summer), should be more cold-resistant than those from mild environments. Our results showed that *A. platensis* populations from Pinto locality characterized as the coldest geographic locality among the latitudinal gradient (**see Chapters 2 and 3**), showed the highest $CT_{Min}$. In addition, parasitoids developing in winter conditions were more resistant to cold than those developing in warmer conditions. These results may be attributed either to local adaptation due to genetic changes or to phenotypic plasticity (acclimation). The difference in thermal tolerance ($CT_{Min1}$ and $CT_{Min2}$) between the tested conditions (**see Chapter 3**) could result from differences in developmental time and body size, as individuals which emerged from winter conditions tolerated better the low temperatures (**Chapter 3**). Acclimating individuals to various environmental temperatures showed that phenotypic plasticity can play a prominent role in coping with thermal stress in aphid parasitoids (Levie et al. 2005). Acclimation produced changes in water and fat content (Lee 1991). It has been observed that cold tolerance is based on increased water content which remains unfrozen in many cold hardened insects, allowing basal metabolism to continue at a low level (Block 2003; Sinclair et al. 2003; Colinet et al. 2006). In the same way, insects adjust their fat reserve and composition to environmental conditions to stabilize detrimental thermal effects (Sinclair 2015; Sinclair and Marshall 2018). Similar results have been reported previously for other parasitoid species (Colinet et al. 2006, 2007c, a, b, 2010, 2015; Colinet and Hance 2010; Colinet and Boivin 2011) which could explain the prevalence of *A. platensis* in winter throughout the central south of Chile during winter.

According to the thermal evolution model, the reduced gene flow between populations due to reproductive isolation may favor local adaptation at different areas on a latitudinal gradient in parasitoids (Angilletta Jr 2009; Tougeron et al. 2019a). Based on the critical thermal tolerance parameters measured ($CT_{Min}$) (**see Chapters 3**), our findings were consistent with previous studies showing that cold temperature tolerance in *Drosophila melanogaster* (Diptera, Drosophilidae) might be locally adapted to native temperatures. This was observed in a study of heritable clinal variation involving more than 80 strains from 17 populations in the Eastern of Australia, where cold latitudinal strains recovered faster from chill coma (i.e., the time taken to recover after a cold shock) than warm strains (Hoffmann et al. 2002). In consequence, a strong selection for genomic variants underlies local adaptation, translating this genetic difference to variation in phenotypes and fitness within natural populations (reviewed by Adrion et al. 2015). The genetic differentiation over small distances could explain our results. For instance, it has been observed that populations of *Diaeretiella rapae* (Hymenoptera, Braconidae) were found to be genetically subdivided at a small range (less than 1 Km) explained by a relatively low dispersal rate (Vaughn and Antolin 1998). One pattern emerging from this study is that short-term exposure measurements (critical thermal limits) typically correlates with the climate of different population origins, coinciding with the local temperature conditions (e.g., total hours below 0° C). These findings are in agreement with the climatic variability hypothesis (Janzen 1967), in which populations at cold environments display broader tolerance ranges than populations inhabiting warmer environments (Kellermann and van Heerwaarden 2019). This suggests that the association between thermal tolerance and the minimum temperature of origin area plays an important role on distribution of aphid parasitoids, improving their fitness on their local environment (Kawecki and Ebert 2004). In summary, the results support the idea that the critical thermal limits (CTL) have evolved in response to local minimal temperatures and that they allow parasitoids to distribute over a range of different climatic conditions within the range tested in this study (**Chapter 2**). During winter, active parasitoids that develop at low temperatures are probably submitted to repeated cold stresses, so the capacity to recover rapidly is an advantage which is correlated to better survival and reproduction.

## Diapause expression: effects on parasitoid community and food-web structure in a climatic gradient

For ectothermic species, such as insects, winter temperature has long been recognized as a major environmental factor responsible for species abundance and geographic distribution. The food-web composition of the aphid–parasitoid species interactions in the central south valley of Chile were dominated mainly by the aphid *R. padi* and its parasitoid *A. platensis.* Other parasitoids *A. ervi, A. uzbekistanicus/rhopalosiphi* also constituted a large proportion of the parasitoids across the geographical range and were mainly associated to the aphids *S. avenae* and *M. dirhodum* but in low abundances, without variation for the food-web metrics analyzed (i.e., Shannon's diversity of interactions, Connectance, Web Asymmetry, the level of specialization (H2), Generality, and Vulnerability). This suggests no strong climatic filtering differences at warm, mild and colder winter areas within the range of winter conditions found in Chile, which does not act upon the aphid-parasitoid trophic networks. In fact, in our study, minimal temperatures and hours below 0° C were not good predictors of aphids and parasitoids abundance (**Chapter 2**). However, our study showed that network structure and food web composition changes for aphid and parasitoids, as their relative abundances vary among the climatic areas in the central south valley of Chile (**Chapter 2**). This is the first time that the aphid-parasitoids food-web in cereals was described in winter in Chile. The causes of the variations of the relative abundances between species are intriguing. The lack of differences found along the latitudinal gradient was probably due to the high frequency of interactions among parasitoid and aphid species, affecting the interaction levels since both aphids and their parasitoids are frequently active in winter with low levels of diapause in cereal crops. This pattern was demonstrated in some countries with mild winter climates (Andrade et al. 2015, 2016; Tougeron et al. 2017, 2018a) with significant effects on food-web functioning. For instance, for the parasitoid community of cereal aphids in western France, diapause expression of *Aphidius avenae* decreased with the increase of temperature in winter (Tougeron et al. 2017). As a consequence, this species is now present and active in winter, which was not the case before 2010, affecting the abundances of other parasitoid species such as *Aphidius rhopalosiphi* and *Aphidius matricariae* recorded previously as dominant species (Andrade et al. 2016). This is due to some niche overlap. Therefore, competition among adult parasitoids of different species may be happening along the climatic gradient in Chile, since it has been demonstrated that parasitoids adopt generalist strategies in response to a shortage of their preferred hosts (Andrade et al. 2016; Ecoche-Bosy et al. 2016; Ortiz-Martínez et al. 2019). Many parasitoid communities have been shown to enhance pest suppression by a complementary use of aphid host resource through niche partitioning suggesting that parasitoids adjust their attack rates on each aphid species according to the overall mean availability of aphids (Tougeron et al. 2018a; Ortiz-Martínez et al. 2019; Tougeron et al. 2020a). Our results showed that winter food webs were compartmentalized through bottom-up effects, as parasitoid abundances were directly explained by aphid abundances. Similar results were observed in aphid-parasitoid networks in cereal fields of

Western France, where the abundances of the different species of aphids determined the abundance of parasitoid species in winter (Andrade et al. 2016; Tougeron et al. 2018a, 2020a).

In addition, interestingly, winter conditions allow the development and activity of hyperparasitoids species in Chile (i.e., *Alloxysta* sp, *Dendrocerus* sp, *Asaphes* sp) (**Chapter 2**). This has also been observed in other aphid-parasitoid communities in which hyperparasitoids are displaying a high parasitism pressure on the primary parasitoids on cereal crops in the west of France (Andrade et al. 2016; Tougeron et al. 2017). Also, we provided the first evidence of one hyperparasitoid species *Phaenoglyphis villosa* expressing diapause along the latitudinal gradient in the central-south valley of Chile.

In summary, our results provide a better understanding of the activity and abundance of parasitoids during winter along a latitudinal gradient in Chile, where winters are mild. These findings suggest a weak effect of mild winter conditions on aphid and parasitoid composition. Food-web structures in the latitudinal gradient supported a high number of species, suggesting that overwintering parasitoid populations/species were active with a bottom-up effect (**Chapter 2**). Therefore, network structure could simply emerge from random encounters among parasitoids and their aphid hosts with a major influence of aphid abundances as the main driver of aphid-parasitoid interactions.

## How does host quality affect diapause expression in aphid parasitoids?

Although it has been recognized that winter diapause expression is regulated mainly by photoperiod and temperature as well as their interactions in aphid parasitoids (Tauber et al. 1986; Brodeur and McNeil 1989; Langer and Hance 2000; Tougeron et al. 2017; Tougeron 2019; Tougeron et al. 2019a), changes in host quality such as species, size and abundance over time may act as a selective pressure, which could as well affect diapause expression (Brodeur and McNeil 1989). Interactions among the generalist parasitoid species *A. ervi* and their aphid hosts *S. avenae* and *A. pisum* represent an excellent model to study different patterns on fitness traits (Zepeda-Paulo et al. 2013, 2015) including diapause (Christiansen-Weniger and Hardie 1999; Tougeron et al. 2017, 2018b, 2019a). The parasitoid *A. ervi* is the predominant parasitoid species controlling *A. pisum* and *S. avenae* although these aphids' species differ in several important aspects (e.g., plant host range and body size) (Daza-Bustamante et al. 2002).

Diapause in *A. ervi* may be mediated by the aphid host under low temperatures and short photoperiod conditions (**Chapter 4**). In this study *A. ervi* entered prepupal diapause if they developed on the pea aphid *A. pisum* at greater frequencies (20%) when compared to parasitoids developing on the grain aphid *S. avenae* (5%). The same pattern was observed in *A. ervi* populations of the west of France where *A. ervi* had a high frequency of diapause when developed in the pea aphid *A. pisum* (Tougeron et al. 2019b) whereas diapause expression was less frequent when *A. ervi* developed in *S. avenae* (Tougeron et al. 2017). One explanation for the higher diapause rate, is that larger aphids can offer better resources under an unfavorable environment, since the preference of female parasitoids is based on the rate of resource acquisition (Barrette et al. 2009). In addition, reserves must be sequestered to survive the diapause period and allow post-diapause development (Hahn and Denlinger 2010). Therefore, a high-quality host guarantees survival and reduces the mortality rate of parasitoids in stochastic environments (Chapter 4). In consequence, parasitoids may suffer a scarcity of hosts in cereal crops at the beginning of the winter in Chile, so that *A. ervi* could use this signal as a stimulus to induced dispersal and then express diapause over aphids present in perennial legumes during this period. This suggests that parasitoids attacking different host aphid species exhibit greater plasticity in diapause induction consistent with the existence of mixed strategies in a population (Menu et al. 2000).

Parasitoids have evolved a diversity of physiological adaptations to survive within a given range of environmental conditions (Hance et al. 2007). Patterns of host use by parasitoids are predicted to be based on both the suitability and abundance of hosts responding to environmental variability through the seasons (Vercken et al. 2015). Effects of photoperiod and temperature on diapause induction have been studied to a certain extent in some aphid parasitoids species (Brodeur and McNeil 1989; Christiansen-Weniger and Hardie 1999; Langer and Hance 2000; Tougeron et al. 2017, 2019b, a, 2020b), but little work has been done on whether biotic cues may act similarly on winter and summer diapause (see Tougeron et al. 2018b). As discussed in the **Chapter 4**, our findings showed the host density effect on winter diapause in aphid parasitoids, but not for summer diapause. The higher diapause incidence at low host density and in winter like laboratory conditions could be interpreted as a response to host shortage in winter as well as a density-dependent reaction process to avoid intraspecific aggression (superparasitism). Indeed, parasitoids adjust their oviposition strategy in response to host density through increasing parasitism and decreasing superparasitism (He and Wang 2008). By contrast, and similar to what was observed by Tougeron et al. (2018b) in other parasitoids species (*A. rhopalosiphi* and *A. avenae*), summer diapause in *A. ervi* was not affected by host resource scarcity, probably because in summer *A. ervi* could find other species to attack. This suggests that host density may potentially modify the interaction between parasitoids and aphids according to seasonality.

In addition to limited host resources, it has been proposed that female competition may act as a selecting factor inducing summer diapause in the progeny to avoid future competition (Tougeron et al. 2018b). Diapause may thus be induced through maternal effects according to the potential state of the environment at the next generation (Vercken et al. 2015). In our experiments, we observed a clear effect of female competition on summer diapause induction in the offspring of *A. ervi* but not in winter conditions. These findings suggest that parasitoids may respond in a different way to the risk of intraspecific competition in summer than in winter (**Chapter 4**; Tougeron et al. 2018b). This response in summer and winter condition may be supported by the way of producing diapause offspring by parasitoids. It has been shown that in some species of *Aphidius*, mothers produce diapausing progeny only in a part of their offspring, others producing only diapause progeny, and other mothers may produce a mix of both (Tougeron et al. 2018b). Therefore, parasitoids may use an adaptive 'bet-hedging' strategy, where the proportion of parasitoids entering diapause should match the probability of a parasitoid undergoing unpredictable summers (Menu and Debouzie 1993; Le Lann et al. 2021). In consequence, cues acting in the maternal generation are expressed as phenotypic differences in the offspring and this response may vary according to the seasonality.

## Implications for biological control

*Aphidius* species, a large group of hymenopteran insects, are well known biological control agents of aphid pests in different agroecosystems (Godfray 1994) and their performance is strongly regulated by environmental temperature (Hance et al. 2007). In aphid parasitoids, the optimal effectiveness of diapause and its synchronization with their host life cycle may influence parasitoid success which may be critical for their impact on aphid pest populations. Understanding how local and large-scale factors affect host-parasitoids interactions is a key challenge in biological pest control programs (Raymond et al. 2016). The mechanisms of success and failure of aphid biological control using parasitoids are not well understood yet, probably because of the lack of knowledge on the life-history strategies involved (Liu et al. 2016).

Our study provided insights of the parasitoid food web composition and their strategies to cope with harsh conditions along the latitudinal gradient in Chile (see discussion **Chapters 2, 3 and 4**). It has been demonstrated that temperature has a strong influence on the diapause expression during winter (Tougeron et al. 2017). The low diapause incidence recorded in aphid parasitoids in Chile is in concordance with other studies that have shown low levels of diapause expression in other regions with mild winters (see Tougeron et al. 2017, 2018c, a, 2020a). Mild winter temperatures could be favorable for increased parasitoid populations that depend on the availability of suitable hosts over the winter period (Tougeron et al. 2017). This result suggests the possibility of manipulating the agroecosystem to increase parasitoid populations using protective elements in the landscape as recommended by Tougeron et al. (2016), providing alternative hosts and adequate microclimate. Remaining active in winter could favor parasitoid species as biological control agents based on the parasitoids naturally present against aphid pests in cereal fields from Chile. Moreover, acclimation could play an important role in the mass production of parasitoids since thermal tolerance could be improved through prior acclimation (**Chapter 3**). In addition, the interest of knowing the winter strategy of parasitoid species can help to propose new biological control programs against other aphid species by building a winter population of parasitoids through habitat manipulation (Alvarez-Baca et al. *in prep*). The optimal temperature for cold storage should be based upon the need to reduce the risk of accumulated chilling damages (Colinet et al. 2007c; Colinet and Hance 2010). On the other hand, the variability of stimuli that induce diapause in parasitoids may guarantee the optimal exploitation of the available resources and sufficient numbers of offspring surviving environmental harsh conditions according to seasonality (**Chapter 4**). Successful adaptations to different environments should involve both local adaptations by genetic changes in diapause expression and plastic responses, including maternal effects (**Chapters 3 and 4,** Tougeron et al. 2018b) which could have an epigenetic response. Mothers are sensitive to cues inducing diapause affecting the phenotype of their offspring based on environmental conditions they experienced, providing significant fitness benefits if future conditions match the ones currently experienced by mothers (Burggren 2017; Reynolds 2017)

Modifications in overwintering strategies in both pests and natural enemies may disrupt food web composition and modify positively or negatively the effectiveness of biological control agents, due to increasing intraguild competition (niche overlapping) (Tylianakis and Binzer 2014; Andrade et al. 2016). However, the mild winter conditions in the central south valley of Chile could provide enough favorable hosts to maintain the richness and/or abundance of parasitoids. This may improve the efficiency of biological control, if niche partitioning exists, promoting the coexistence of different parasitoids species whilst exploiting the same resources (Outreman et al. 2018). This suggests that quite low environmental filtering mediated by the climatic gradient through parasitoids contrasting traits might represent the dominant mechanism in structuring aphid-parasitoid assemblages in cereal crops. Therefore, understanding the mechanism that facilitates the co-existence of competing parasitoid species is fundamental for developing and implementing biological control programs against aphid pests (Raymond et al. 2016; Ortiz-Martínez et al. 2019). Increasing the number of active parasitoids could enhance natural biological control of aphids through increased parasitism rates (Hance et al. 2007; Tougeron et al. 2017). Indeed, the high level of interactions between aphids and their parasitoids observed in this study is fundamental for the effectiveness of biological control strategies. Nevertheless, aphids and parasitoids are not alone and the presence of non-diapausing hyperparasitoids recorded in the central-south valley of Chile (**Chapter 2**) may reduce the efficiency of primary parasitoids biological control in the fields (Tougeron et al. 2018a; Tougeron and Tena 2019). Therefore, it might be possible to implement national-scale biological control strategies such as Augmentative biological control for cereal aphids, since the species composition and abundance are similar in communities along the climatic gradient.

## Perspectives and future directions

While abiotic stressors, including extreme temperatures, are common in nature, they are also a serious problem for agriculture. Low and high temperatures are considered to be some of the major sources of abiotic stress for pests and natural enemies (Langer and Hance 2000; Hance et al. 2007; Kellermann and van Heerwaarden 2019). The phenotypic expression of diapause depends on the genotype, environmental influences, and indirect genetic effects such as maternal effects. In this study, we observed that entering in diapause or not, for an aphid parasitoid, is influenced by different environmental cues. Therefore, the plasticity of individuals may ensure that at least part of the population will survive in a stochastic environment. These results highlight the need for further studies to evaluate the diapause in aphid parasitoids in a clear climatic gradient. In addition, it is necessary understand the mechanisms by which diapause is initiated in one part of the population and not in another, in species that express diapause partially (e.g., *A. ervi*), its genetic basis, and the evolution of this trait, which is critically important for the insect's life histories.

In temperate regions, the recurring seasonal conditions represent a fundamental physiological challenge for a wide range of insects. In response, many temperate insects enter diapause, an alternative physiological trait characterized by altered gene expression profiles, which mediate developmental arrest, metabolic depression, increased stress resistance, and other physiological changes (Tougeron 2019), allowing organisms to synchronize their life cycle with the seasonal environmental variations (Denlinger 2002). Although this work has identified different biotic and abiotic cues influencing diapause induction in parasitoids as well as plastic responses (acclimation) on the cold tolerance phenotypes and their effects on the community composition along the latitudinal gradient in Chile, it has not been possible to ascertain what mechanisms are underpinning this response. This raises questions about the mechanisms and costs of such adaptation and about the species ability to evolve to new conditions, which will be important for predicting the evolutionary and plastic responses (including epigenetic mechanisms) of aphid parasitoids in response to climate change. Epigenetic mechanisms such as DNA methylation and histone modifications have been identified as essentials for the phenotypic plasticity of insects in their interaction with the environment (Burggren 2017). In insect, diapause maybe categorized into three different phases: Preparation, maintenance and termination (Koštál 2006); however, the molecular signals of the mechanisms driving development through these phases are only partly understood. Hence, the gene expression that influences diapause would be interesting to investigate.

On the other hand, insects have the capacity to adjust their physiological mechanisms during their lifetime to promote thermal tolerance and cope with sublethal conditions, a phenomenon referred to as thermal acclimation (Enriquez and Colinet 2019). However, mechanisms underlying physiological and molecular responses or acclimation to cold and heat stress are still largely unknown. The expression of up-regulated and down-regulated genes in cold acclimated flies *Drosophila suzukii* have been identified (Enriquez and Colinet 2019), where many factors can be involved in insect cold hardening (Clark and Worland 2008). Cryoprotectants are used to maintain a liquid intracellular space and protect membrane bilayer structure in the cells. The best-known mechanisms of insect cold hardiness are carbohydrate cryoprotectants, AFPs and INAs or INPs (Clark et al. 2009; Storey and Storey 2012). In the same way, freeze-tolerant species may also produce antifreeze proteins along with ice nucleating proteins (Clark and Worland 2008). Ice nucleators have also been identified in extracellular matrices (ECM) of freeze-tolerant invertebrates. On the other hand, heat shock proteins (HSPs) and other chaperones have been identified as part of the cell preservation strategy for both cold and heat survival (Clark and Worland 2008; Sørensen et al. 2019). HSPs promote to cold tolerance and improve to repair chill injuries (Sorensen et al. 2001). In parasitoids, developmental acclimation generally leads to a beneficial effect on cold tolerance, with an improvement of the critical thermal minimal ($CT_{Min}$) (**Chapter 3**). Therefore, these mechanisms could explain the higher cold tolerance of the different species of aphid parasitoids when facing different climatic events (i.e., harsh winters), which is why their study is essential. Parasitoids can display high and efficient cold tolerance plasticity; this capability likely contributes to their invasive success in temperate cold regions.

In this thesis, we identified the role of the maternal effect as a signal inducing diapause in aphid parasitoids differently in summer and winter conditions. For this reason, maternal effects identified here (**Chapter 4**) and other transgenerational effects that increase the diapause expression (Tougeron et al. 2018b, 2020b), could be associated with epigenetic mechanisms. Epigenetic molecular mechanisms are important for numerous functions in many organisms, regulating developmental, ontogenetic trajectories, and environmental adaptations (Villagra and Frías-Lasserre 2020) and could be induced by conditions experienced by the maternal generation (Reznik et al. 2012). Therefore, future studies could use different techniques such as RNA interference to evaluate if DNA methylation is correlated with gene expression during diapause. Which providing us the opportunity of identifying an ecologically relevant epigenetic modification in diapause induction of the aphid parasitoids.

With the increasing availability of insect genomic resources and the number of completed genomes, the genomic resources available for parasitoids researchers' community are growing (Dennis et al. 2020). This information could be used to explain changes in seasonal phenology, development under different environmental conditions and what mechanisms are involved in inducing and terminating diapause.

Understanding ecological and evolutionary responses of parasitoids to complex interacting factors linked to environmental change is a significant challenge that needs to be studied to get the ability to predict parasitoid dynamics and to anticipate the management of agroecosystems at different scales. To cope with increasingly fluctuating and unpredictable environments, parasitoid species can either evolve through rapid local adaptation and /or plasticity responses (**Chapters 2, 3 and 4**). In Chile, it has been projected that minimum and maximum temperatures will increase in the entire territory throughout all seasons (Araya-Osses et al. 2020). The future research of the impacts of climate change on insect pests and their natural enemies must concentrate on identifying the possible loss of synchrony between aphids and parasitoids in different crops, since the impacts of climate changes will be mitigated moving crops to new areas in Chile (Melo and Foster 2021). Therefore, it will be essential to follow the whole aphid-parasitoid community in the coming years. Additionally, the need to include hyperparasitoids in future studies is essential as part of biological control programs as hyperparasitoids may have huge potential to disrupt biological control in different agroecosystems (Tougeron and Tena 2019).

Therefore, aphid parasitoids have proved to be an ideal model insect for studying diapause and other strategies to cope with variable environments. Within this Ph.D. thesis, our combination of laboratory and field-based studies has identified that parasitoids have proved to be a useful guild in further substantiating the link between environmental conditions, diapause, and the implementation of cold tolerance mechanisms. However, would be useful to evaluate the overwintering strategies in aphid parasitoids on a clear climatic gradient with areas where colder winters occur.

## General conclusion

A trait-based understanding of temperature effects on parasitoids is important as it allows us to identify the types of strategies that facilitate or limit the species coexistence in thermally variable environments. In summary, because the geographical range of aphid parasitoid species covers a broad area in the central—south valley of Chile, we expected to find diapause differentiation in natural populations (**Part 1**). The results of this Ph. D research offer new insights into the effects of the cues inducing diapause in parasitoids. No latitudinal gradient in temperatures was recorded in the studied area, even if variation in minimal temperatures and the number of hours below 0°C may generate a transversal gradient (**Parts 2 and 3**). Also, thermal acclimation and local adaptation determine the fate of species facing environmental variability (**Part 3**) showing climatically determined responses.

In addition to abiotic factors (photoperiod and temperature), diapause induction seems to arise from biotic factors. In this study, we provide evidence that diapause in parasitoids was influenced by the attacked host species and from the perception of host scarcity in winter diapause. Likewise, direct intraspecific competition among parasitoid females in summer conditions has been translated into increase of the diapause offspring, however, this effect was not observed in winter conditions (**Part 3**). Therefore, modifications in parasitoid community composition are linked to shifts in diapause expression. Thus, developmental plasticity and the observed evolutionary responses offer a mechanistic explanation for adaptive life-history variation in aphid parasitoids with implications in aphid parasitoid interactions suggesting that the success of parasitoids as natural agents depends strongly on host density and timing of seasonal activities.

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# Estrategias de hibernación de un gremio de parasitoides de áfidos de los cereales a lo largo de un gradiente latitudinal

Comprender cómo el clima influye en la regulación de las poblaciones de insectos a través de las interacciones entre especies es una pregunta muy importante en ecología. Para los ectotermos como los insectos, la temperatura es reconocida como un factor ambiental importante responsable de la abundancia y distribución geográfica de las especies. Los sistemas hospedero-parasitoide se encuentran entre las interacciones ecológicas más importantes afectadas por los cambios ambientales debido a la posición trófica elevada de los parasitoides, lo que puede conducir a diferencias en las respuestas fenológicas (estrategias estacionales) y cambios de distribución (Godfray 1994; Bale and Hayward 2010; Kehoe et al. 2018; Tougeron et al. 2020a). La diapausa es una adaptación que permite a los parasitoides sincronizarse con los cambios estacionales y sus hospederos. (Polgár and Hardie 2000). Durante esta tesis, se exploran varios impulsores ecológicos en un gremio de parasitoides de áfidos de los cereales a lo largo de un gradiente latitudinal en el valle centro-sur de Chile. Se evalua si las condiciones ambientales a lo largo de este gradiente conducen a una mayor incidencia de diapausa en latitudes altas (más frías) en comparación con latitudes bajas (más cálidas). Además, exploramos si la diapausa puede estar regulada por factores bióticos (es decir, el efecto de la especie hospedera, la disponibilidad de los hospederos y la competencia intraespecífica entre hembras) además de factores abióticos (fotoperíodo y temperatura), utilizando enfoques de laboratorio y de campo.

Los parasitoides de áfidos y sus hospederos son organismos ampliamente distribuidos que viven en diferentes condiciones climáticas. Los parasitoides son los principales agentes de regulación biológica de las poblaciones de áfidos en diferentes agroecosistemas (Godfray 1994). Por consiguiente, la variabilidad de las poblaciones de parasitoides afecta la regulación de plagas de áfidos (Neil and Jervis 2007). La amplia distribución geográfica de esta interacción biótica la convierte en un modelo interesante para el estudio a escala continental. Las condiciones ambientales pueden afectar la distribución, la fenología, la fecundidad y las estrategias de hibernación de las especies de parasitoides con efectos en la composición de la comunidad y la fuerza de las interacciones bióticas. Por lo general, las especies de parasitoides están expuestas a temperaturas estresantes en su entorno natural debido a los ciclos estacionales. Al igual que otros ectotermos, los parasitoides tienen una capacidad limitada para regular su temperatura corporal, por lo tanto, han desarrollado una variedad de estrategias para sobrevivir en ambientes térmicamente estresantes, ya sea a través de la aclimatación (plasticidad) o la adaptación genética. Aquí presentamos un marco para comprender los efectos de los factores abióticos y bióticos en las estrategias estacionales de los parasitoides que incorpora una descripción mecánica de las respuestas de los rasgos de la historia de vida que caracterizan la fenología de muchos ectotermos.

En función de la intensidad del invierno, las localidades muestreadas se clasificaron en zonas con inviernos fríos, templados o cálidos, considerando su variabilidad interanual, a lo largo del gradiente latitudinal en el valle centro-sur de Chile. Nuestra primera hipótesis fue que la incidencia de la diapausa variaría a lo largo del gradiente latitudinal con una mayor incidencia de diapausa en las zonas más frías. En consecuencia, una disminución de la actividad de los parasitoides en las zonas más frías durante el período invernal seria observada, afectando la composición y estructura de la red. Sin embargo, nuestros resultados no lograron demostrar un gradiente latitudinal claro en las tasas de diapausa, ya que obtuvimos niveles bajos de incidencia de diapausa para todas las poblaciones estudiadas (aproximadamente 4 %), probablemente debido a que las áreas más frías no son lo suficientemente frías como para inducir la diapausa. Por lo tanto, la evidencia disponible no apoya la hipótesis de que la diapausa en los parasitoides aumenta con el aumento de la latitud en el valle centro-sur de Chile. Se observó niveles bajos de diapausa solo en tres de las nueve especies de parasitoides registradas, *Aphidius ervi*, *A uzbekistanicus* y *Praon volucre* y, curiosamente, una especie de hiperparasitoide *Phaenoglyphis villosa*. Consecuentemente, las interacciones hospedero-parasitoide en cultivos de cereales a lo largo del gradiente no difirieron en la composición de la red alimentaria. Las diferencias observadas para las tres áreas estudiadas se refieren únicamente a la abundancia relativa de especies dentro de los gremios, con variaciones espaciales y temporales, principalmente para las especies menos abundantes. Estos resultados fueron confirmados por nuestros experimentos de laboratorio donde no se observó diapausa en *A. platensis* para ninguna de las poblaciones estudiadas y con bajos niveles de diapausa para la especie *A. ervi* (alrededor del 5 %), incluso cuando estas poblaciones estuvieron sujetas a condiciones invernales que indujeron la diapausa en otras poblaciones de parasitoides de áfidos. Los individuos dentro del gremio de parasitoides de áfidos están activos durante el invierno, lo que sugiere que la estrategia de la mayoría de los individuos en cualquier población es pasar el invierno como adultos activos. La ausencia y/o los bajos niveles de diapausa observados pueden deberse a las condiciones invernales insuficientemente frías en el valle centro-sur de Chile. Estudios recientes señalan que este valle viene sufriendo una relativa homogeneización interanual de temperaturas debido al cambio climático (Falvey and Garreaud 2009; Mutz et al. 2021). En el noroeste de Francia, tras el calentamiento invernal, se ha observado una disminución de la expresión de diapausa en determinadas poblaciones de parasitoides en condiciones naturales y de laboratorio. (Tougeron et al. 2017, 2018a, 2020a). Contrariamente a nuestros resultados, algunos estudios que utilizan otras especies de parasitoides como *Nasonia vitripennis* Ashmead (Hymenoptera: Pteromalidae) y *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) como modelos, mostraron altos niveles de incidencia de diapausa en latitudes caracterizadas por condiciones climáticas severas en el norte de Europa en comparación con las poblaciones del sur. que experimentan inviernos más suaves (Paolucci et al. 2013; Zhang et al. 2017). Por lo tanto, la baja incidencia de diapausa observada en los parasitoides, podría entenderse como una respuesta a los inviernos templados que se presentan en el valle centro-sur de Chile, que además permiten una continua disponibilidad de hospedantes durante toda la temporada invernal, ya que las especies de áfidos *Rhopalosiphum padi*, *Sitobion avenae* y *Metopolophium dirhodum* estuvieron presentes en los cultivos de cereales durante todo el período. Estudios previos también han registrado áfidos de los cereales anholocíclicos estacionalmente activos en Europa occidental, caracterizados por inviernos templados (Langer et al. 1997; Dedryver et al. 2001; Andrade et al. 2015), lo que permite que algunas especies de parasitoides pasen el invierno como adultos activos. En invierno, los rasgos fisiológicos y de historia de vida varían a lo largo de los gradientes geográficos, permitiendo que los individuos que permanecen activos se enfrenten a diferentes entornos. Entre estos rasgos, la tolerancia térmica juega un papel importante cuando los insectos se enfrentan a condiciones adversas.

(Turnock and Fields 2005; Angilletta Jr 2009). La variación del fotoperíodo y la temperatura a lo largo de los gradientes geográficos debería conducir a la adaptación local de las poblaciones naturales de acuerdo con sus tolerancias térmicas (Begon et al. 2006; Blanquart et al. 2013; Merilä and Hendry 2014). Se predijo que los individuos que se desarrollan en latitudes más frías deberían ser más resistentes al frío que aquellos desarrollados en ambientes templados. Nuestros resultados mostraron que las poblaciones de *A. platensis* de la localidad de Pinto, caracterizada como la localidad geográfica más fría entre las localidades estudiadas, presentó la mayor $CT_{Min}$. Además, los parasitoides que se desarrollaron en condiciones invernales más severas eran más resistentes al frío que los que se desarrollaban en condiciones más cálidas. Estos resultados pueden atribuirse a la adaptación local debido a modificaciones genéticas o a la plasticidad fenotípica (aclimatación). La diferencia en la tolerancia térmica ($CT_{Min1}$ y $CT_{Min2}$) entre las condiciones probadas podría deberse a diferencias en la duración del desarrollo y el tamaño corporal, ya que los individuos que emergieron en condiciones invernales toleraron mejor las bajas temperaturas. La aclimatación de los individuos a diversas temperaturas ambientales ha demostrado que la plasticidad fenotípica puede desempeñar un papel importante en el manejo del estrés térmico en los parasitoides de áfidos (Levie et al. 2005), produciendo cambios en el contenido de agua y lípidos (Lee 1991). Se ha observado que la tolerancia al frío se basa en un mayor contenido de agua que permanece sin congelar en muchos insectos aclimatados al frío. (Block 2003; Sinclair et al. 2003; Colinet et al. 2006). De manera similar, los insectos ajustan sus reservas de lípidos a las condiciones ambientales para estabilizar los efectos adversos del estrés térmico. (Sinclair 2015; Sinclair and Marshall 2018). Estos resultados podrían explicar la prevalencia de *A. platensis* en invierno en todo el centro-sur de Chile. Por otro lado, según el modelo de evolución térmica, la reducción del flujo de genes entre poblaciones debido al aislamiento reproductivo puede promover la adaptación local a diferentes áreas en un gradiente latitudinal en parasitoides. (Angilletta Jr 2009; Tougeron et al. 2019a). En base en la tolerancia térmica medida ($CT_{Min}$), nuestros resultados son consistentes con estudios previos que muestran que la tolerancia al frío podría adaptarse a las temperaturas locales (Hoffmann et al. 2002). En resumen, los resultados respaldan la idea de que los límites térmicos críticos (CTL) evolucionaron en respuesta a las temperaturas mínimas locales y que permiten que los parasitoides se distribuyan en un rango de diferentes condiciones climáticas dentro del rango probado en este estudio. Durante el invierno, los parasitoides activos que prosperan en temperaturas bajas probablemente estén sujetos a estrés térmico por frío repetido y, por lo tanto, la capacidad de recuperarse rápidamente es una ventaja relacionada con una mejor supervivencia y reproducción.

Nuestro trabajo, es el primer estudio en describir las redes alimentarias áfidos de los cereales de invierno y sus parasitoides en Chile. Nuestros resultados muestran que la composición de las redes alimentarias está dominada principalmente por el áfido *R. padi* y su parasitoide *A. platensis*. Otros parasitoides como *A. ervi* y *A. uzbekistanicus/rhopalosiphi* también representan una gran proporción de los parasitoides presentes en el área geográfica estudiada y están asociados principalmente con los pulgones *S. avenae* y *M. dirhodum.* Sin embargo, estas especies se encuentran en baja abundancia, sin que esto afecte los parámetros de la red alimentaria analizados (diversidad de interacción de Shannon, conectividad, asimetría, nivel de especialización (H2), generalidad y vulnerabilidad). Esto sugiere que no existen fuertes diferencias en el filtrado climático en el rango de condiciones invernales observadas en Chile, el cual no actúa sobre las redes tróficas áfido-parasitoide. De hecho, en nuestro estudio, las temperaturas mínimas y las horas por debajo de 0°C no resultaron ser buenos predictores de la abundancia de diferentes especies de áfidos y sus parasitoides. Sin embargo, reveló que la estructura y composición de la red alimenticia de áfidos y parasitoides varió en las abundancias relativas de las diferentes especies según las zonas climáticas del valle central sur de Chile. Se desconocen las causas de las variaciones en la abundancia relativa entre especies. La ausencia de diferencias observadas a lo largo del gradiente latitudinal probablemente se debió a la alta frecuencia de interacciones entre las especies de parasitoides y áfidos. Este patrón también se ha observado en algunos países con climas invernales templados (Andrade et al. 2015, 2016; Tougeron et al. 2017, 2018a) con efectos significativos en el funcionamiento de la red alimentaria. Por ejemplo, para la comunidad de parasitoides de áfidos de cereales en el oeste de Francia, la expresión de diapausa de *Aphidius avenae* disminuyó con el aumento de la temperatura en invierno. (Tougeron et al. 2017). Como resultado, esta especie ahora está presente y activa en invierno, lo que afecta la abundancia de otras especies de parasitoides como *Aphidius rhopalosiphi* y *Aphidius matricariae* previamente registradas como especies dominantes (Andrade et al. 2016). Esto se debe a una superposición de nicho. Por lo tanto, la competencia entre parasitoides adultos de diferentes especies puede ocurrir a lo largo del gradiente climático en Chile, ya que se ha demostrado que los parasitoides adoptan estrategias generalistas en respuesta a la escasez de sus hospedantes preferidos. (Andrade et al. 2016; Ecoche-Bosy et al. 2016; Ortiz-Martínez et al. 2019). Nuestros resultados mostraron que las redes alimentarias de invierno estaban compartimentadas por efectos de abajo hacia arriba, ya que la abundancia de parasitoides se explica directamente por la abundancia de áfidos. Además, las condiciones invernales han permitido el desarrollo y actividad de especies de hiperparasitoides en Chile (*Alloxysta* sp, *Dendrocerus* sp, *Asaphes* sp). Este patrón, también se ha observado en otras comunidades de parasitoides de áfidos en la que los hiperparasitoides ejercen una fuerte presión sobre los parasitoides primarios en cultivos de cereales en el oeste de Francia. (Andrade et al. 2016; Tougeron et al. 2017). Además, proporcionamos la primera observación de una especie de hiperparasitoide (*Phaenoglyphis villosa*) que expresa diapausa a lo largo del gradiente latitudinal en el valle centro-sur de Chile. En resumen, nuestros resultados brindan una mejor comprensión de la actividad y abundancia de los parasitoides durante el invierno a lo largo de un gradiente latitudinal en Chile, donde los inviernos son templados. Estos resultados sugieren un débil efecto de las condiciones invernales sobre la composición de las redes de áfidos y sus parasitoides. La red alimentaria de invierno incluye un gran número de especies. Por lo tanto, la estructura de la red podría surgir simplemente de encuentros aleatorios entre los parasitoides y sus áfidos hospederos con una gran influencia de la abundancia de áfidos como el principal impulsor de las interacciones áfido-parasitoide.

Aunque se ha reconocido que la expresión de la diapausa invernal está regulada principalmente por el fotoperíodo y la temperatura y sus interacciones en los parasitoides de áfidos (Tauber et al. 1986; Brodeur and McNeil 1989; Langer and Hance 2000; Tougeron et al. 2017; Tougeron 2019; Tougeron et al. 2019a), la especie hospedera, el tamaño y sus abundancias relativas pueden representar presiones selectivas, que también podrían afectar la expresión de la diapausa (Brodeur and McNeil 1989). Las interacciones entre la especie de parasitoide generalista *A. ervi* y sus áfidos hospederos *S. avenae* y *A. pisum* representan un modelo excelente para estudiar los rasgos de fitness (Zepeda-Paulo et al. 2013, 2015), incluida la diapausa (Christiansen-Weniger y Hardie 1999; Tougeron et al.2017, 2018b, 2019a). La diapausa en *A. ervi* puede ser mediada por el áfido hospedero en condiciones de baja temperatura y fotoperíodo corto. En este estudio, *A. ervi* entró en diapausa si se desarrollaba sobre el áfido del guisante *A. pisum* a frecuencias más altas (20 %) en comparación con los parasitoides que se desarrollaban sobre el áfido de los cereales *S. avenae* (5 %). El mismo patrón se observó en las poblaciones de *A. ervi* del oeste de Francia, donde *A. ervi* tuvo un alto nivel de diapausa en el áfido del guisante *A. pisum* (Tougeron et al. 2019b), mientras que la expresión de la diapausa fue menos frecuente en *S. avenae* (Tougeron et al. 2017). La mayor tasa de la diapausa en *A. pisum* puede explicarse potencialmente porque los áfidos de esta especie son de mayor tamaño y, por lo tanto, pueden ofrecer mejores recursos en un entorno desfavorable, ya que la preferencia de las hembras por los parasitoides se basa en la tasa de adquisición de recursos. (Barrette et al. 2009). De hecho, las reservas deben acumularse para permitir la supervivencia durante el período de desarrollo de diapausa y postdiapausa. (Hahn and Denlinger 2010). Por consiguiente, un hospedero de alta calidad garantiza la supervivencia y reduce la tasa de mortalidad de los parasitoides en ambientes estocásticos. Los parasitoides probablemente perciben un agotamiento de hospedantes en los cultivos de cereales al inicio del invierno en Chile, por lo que *A. ervi* podría usar esta señal como estímulo para la dispersión y luego expresar diapausa sobre pulgones presentes en leguminosas perennes durante este período. Esto sugiere que los parasitoides que atacan a diferentes especies hospederas de áfidos, exhiben plasticidad en la inducción de diapausa consistente con la existencia de estrategias mixtas en una población (Menu et al. 2000). Los efectos del fotoperíodo y la temperatura en la inducción de la diapausa se han estudiado en algunas especies de parasitoides de áfidos (Brodeur y McNeil 1989; Christiansen-Weniger y Hardie 1999; Langer y Hance 2000; Tougeron et al. 2017, 2019b, a, 2020b), pero se pocos trabajos se han desarrollado para determinar si los factores bióticos pueden actuar de la misma manera en la diapausa de invierno y verano (ver Tougeron et al. 2018b). Nuestros resultados mostraron el efecto de la densidad de hospederos sobre la diapausa de invierno en parasitoides de áfidos, pero no sobre la diapausa de verano. La alta incidencia de diapausa a baja densidad de hospederos y condiciones de laboratorio similares a las del invierno podría interpretarse como una respuesta a la escasez de hospederos de invierno, así como un proceso de retroalimentación dependiente de la densidad para evitar la competencia intraespecífica (que conduce al súper parasitismo). De hecho, los parasitoides ajustan su estrategia de oviposición en respuesta a la densidad del hospedero al limitar el super parasitismo. (He and Wang 2008). Por otro lado, y de manera similar a lo observado por Tougeron et al. (2018b) en otras especies de parasitoides (*A. rhopalosiphi* et *A. avenae*), nuestros resultados muestran que la diapausa de verano en *A. ervi* no se vio afectada por la escasez de hospederos, probablemente porque en verano, *A. ervi* podría encontrar otras especies para atacar. Esto sugiere que la densidad de hospederos puede modificar potencialmente la interacción entre parasitoides y áfidos dependiendo de la estacionalidad. Además de los recursos limitados, se ha propuesto que la competencia femenina puede actuar como un factor de selección que induce la diapausa de verano en la descendencia para evitar una competencia futura (Tougeron et al. 2018b). Por lo tanto, la diapausa puede ser inducida por efectos maternos según el estado potencial del ambiente en la siguiente generación (Vercken et al. 2015). En nuestros experimentos, observamos un claro efecto de la competencia femenina sobre la inducción de la diapausa de verano en *A. ervi* pero no en condiciones de invierno. Estos resultados sugieren que los parasitoides pueden reaccionar de manera diferente al riesgo de competencia intraespecífica en verano que en invierno. (Tougeron et al. 2018b). En algunas especies de *Aphidius*, se ha demostrado que las madres adoptan una estrategia mixta, con solo una parte de su descendencia entrando en diapausa (Tougeron et al. 2018b). En consecuencia, los parasitoides pueden usar una estrategia adaptativa de " "bet-hedging", donde la proporción de parasitoides que entran en diapausa debe coincidir con la probabilidad de que un parasitoide experimente veranos impredecibles. (Menu and Debouzie 1993; Le Lann et al. 2021). Como resultado, las señales que actúan en la generación materna se traducen en diferencias fenotípicas en la descendencia y esta respuesta puede variar según la estacionalidad.

En conclusión, nuestro estudio proporciona información sobre la composición de la red alimentaria de los áfidos de los cereales y sus parasitoides y sus estrategias para hacer frente a las condiciones invernales a lo largo del gradiente latitudinal en Chile. La baja incidencia de diapausa registrada en parasitoides de áfidos en Chile es consistente con otros estudios que han mostrado bajos niveles de expresión de diapausa en otras regiones con inviernos templados (ver Tougeron et al. 2017, 2018c, a, 2020a). Las temperaturas templadas del invierno podrían ser favorables para el aumento de las poblaciones de parasitoides que dependen de la disponibilidad de hospederos durante el período invernal (Tougeron et al. 2017). Este resultado sugiere la posibilidad de manejar el ambiente/hábitat alrededor de las parcelas para aumentar las poblaciones de parasitoides mediante el uso de elementos favorables en el paisaje como lo recomiendan Tougeron et al. (2016) o Damián et al. (2017), proporcionando hospederos alternativos y un microclima adecuado. Mantenerse activo en invierno podría favorecer a las especies de parasitoides como agentes de control biológico de parasitoides naturales contra plagas de áfidos en campos de cereales en Chile. Además, la aclimatación podría jugar un papel importante en la liberación masiva de parasitoides que se producen comercialmente, ya que se podría mejorar la tolerancia térmica mediante una aclimatación previa. Por lo tanto, podría ser posible implementar estrategias de control biológico a nivel nacional, como el control biológico por inundación de los pulgones de los cereales, ya que la composición y abundancia de especies son similares en las comunidades a lo largo del gradiente latitudinal.

Por lo tanto, los parasitoides de áfidos han demostrado ser un modelo biológico ideal para estudiar la diapausa y otras estrategias para hacer frente a entornos variables. Dentro de esta tesis doctoral, nuestra combinación de estudios de laboratorio y de campo, muestran que los parasitoides demostraron ser un gremio útil para corroborar aún más el vínculo entre las condiciones ambientales, la diapausa y la implementación de mecanismos de tolerancia al frío. Sin embargo, sería útil evaluar las estrategias de hibernación en parasitoides de áfidos en un claro gradiente climático con áreas donde ocurren inviernos más fríos.

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# Publications and meetings

***International peer-reviewed journals.***

2021. Armando Alfaro-Tapia, Jeniffer K. Alvarez-Baca, Christian C Figueroa, and Eduardo Fuentes-Contreras, Sub-Lethal Effects of λ-Cyhalothrin on Behavior and Development of the Parasitoid *Aphidius colemani* (Hymenoptera: Braconidae) on kdr-Resistant and Susceptible Green Peach Aphid, *Myzus persicae* (Hemiptera: Aphididae), *Journal of Economic Entomology*, toab147, https://doi.org/10.1093/jee/toab147

2021. Armando Alfaro-Tapia, Jeniffer K. Alvarez-Baca, Kevin Tougeron, Blas Lavandero, Cécile Le Lann and Joan van Baaren. Overwintering strategies and life-history traits of different populations of *Aphidius platensis* along a latitudinal gradient in Chile. *Entomologia Generalis*, (in press) https://doi:10.1127/entomologia/2021/1186

2021. Armando Alfaro-Tapia, Jeniffer K. Alvarez-Baca, Eduardo Fuentes-Contreras, and Christian C. Figueroa. Biological Control May Fail on Pests Applied with High Doses of Insecticides: Effects of Sub-Lethal Concentrations of a Pyrethroid on the Host-Searching Behavior of the Aphid Parasitoid *Aphidius colemani* (Hymenoptera, Braconidae) on Aphid Pests, *Agriculture*, 11(6), 539; https://doi.org/10.3390/agriculture11060539

2020. Jeniffer K. Alvarez-Baca, Armando Alfaro-Tapia, Blas Lavandero, Cécile Le Lann and Joan Van Baaren. Suitability and profitability of a cereal aphid for the parasitoid *Aphidius platensis* in the context of conservation biological control of *Myzus persicae* in orchards. *Insects*, 11(6), 381; doi: https://doi.org/10.3390/insects11060381

2019. Ainara Peñalver-Cruz, Jeniffer K. Alvarez-Baca, Armando Alfaro-Tapia, Leandro M. Gontijo, and Blas Lavandero. Manipulation of Agricultural Habitats to Improve Conservation Biological Control in South America. *Neotropical Entomology* 48, 875–898. https://doi.org/10.1007/s13744-019-00725-1

***Submitted to international peer-reviewed journals***

2021. Armando Alfaro-Tapia, Jeniffer K. Alvarez-Baca, Kévin Tougeron, Joan van Baaren, Blas Lavandero, and Le Lann C. Composition and structure of winter aphid–parasitoid food webs along a latitudinal gradient in Chile. *Oecology* (under revision).

***In preparation to submitted to international peer-reviewed journals***

Armando Alfaro-Tapia, Joan van Baaren, Cécile Le Lann, and Blas Lavandero. *Biotic and abiotic factors inducing winter and summer diapause in Aphidius ervi.*

Jeniffer K. Alvarez-Baca, Xiomara Montealegre, Armando Alfaro-Tapia, Blas Lavandero, Cécile Le Lann and Joan Van Baaren. *Cereal cover crops do not alter the food web structure in aphid–parasitoid interaction on plum orchards.*

Alfaro-Tapia, A. and C. Rasmussen. *Diversity of bees (Hymenoptera, Apoidea) in an altitudinal gradient of the Manu National Park, Peru.*

***Annual meetings***

2021. Armando Alfaro-Tapia, Cécile Le Lann and Joan van Baaren and Blas Lavandero. Effects of host density and maternal competition on diapause in an aphid parasitoid. *The 1st International Electronic Conference on Entomology*.

2020. Armando Alfaro-Tapia, Jeniffer K. Alvarez-Baca, Kevin Tougeron, Blas Lavandero, Cécile Le Lann and Joan van Baaren. Quantifying the aphid-parasitoids food-web structure in cereal crops along a latitudinal gradient. *Entomology 2020-ESA's virtual annual meeting*, Entomological Society of America.

2020. Jeniffer K. Alvarez-Baca, Armando Alfaro-Tapia, Xiomara Montealegre, Blas Lavandero, Cécile Le Lann and Joan Van Baaren. Effect of the cover crops on the natural enemies' assemblage on plum orchards. *Entomology 2020-ESA's virtual annual meeting*, Entomological Society of America.

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2018. Armando Alfaro-Tapia, Jeniffer K. Alvarez-Baca, Kevin Tougeron, Blas Lavandero, Cécile Le Lann, Joan van Baaren. Overwintering strategy of different populations of *Aphidius colemani* from Chile. *SFECOLOGIE-International Conference on Ecological Sciences*, FRANCIA, Rennes.

2018. Jeniffer K. Alvarez-Baca, Blas Lavandenro, Cécile Le Lann, Armando Alfaro-Tapia, van Baaren, Joan. Host suitability for *Aphidius colemani* (Hymenoptera: Braconidae): Can host origin influence parasitoid's choice and progeny fitness? *SFECOLOGIE-International Conference on Ecological Sciences*, FRANCIA, Rennes